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Benzylic hydroxylations of the Amaryllidaceae alkaloids

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by

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INTRODUCTION

Many investigations have been carried out in the last 10 years to determine the biosynthetic pathways of the Amaryllidaceae alkaloids. The origins of the C_6-C_1 and C_6-C_2 units of these alkaloids from the amino acid pool have been delineated. Oxidation and cyclization of norbelladine-type precursors have provided the unifying concept to biosynthetically interrelate the dissimilar ring systems found in the alkaloids.

This thesis is concerned with the synthesis of 3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride and its N-methyl analog in order to carry out tracer studies aimed at their possible roles as C_6-C_1 precursors.

Because there is increasing interest in the sequence and stereochemistry of hydroxylations in the late-stage modifications of many alkaloids, a second section deals with the synthesis of O-methyl[1'R-^{3}H,1-^{14}C]norbelladine in order to examine the stereochemistry of the hydroxylation at the benzylic carbon atom alpha to the nitrogen atom in these alkaloids.

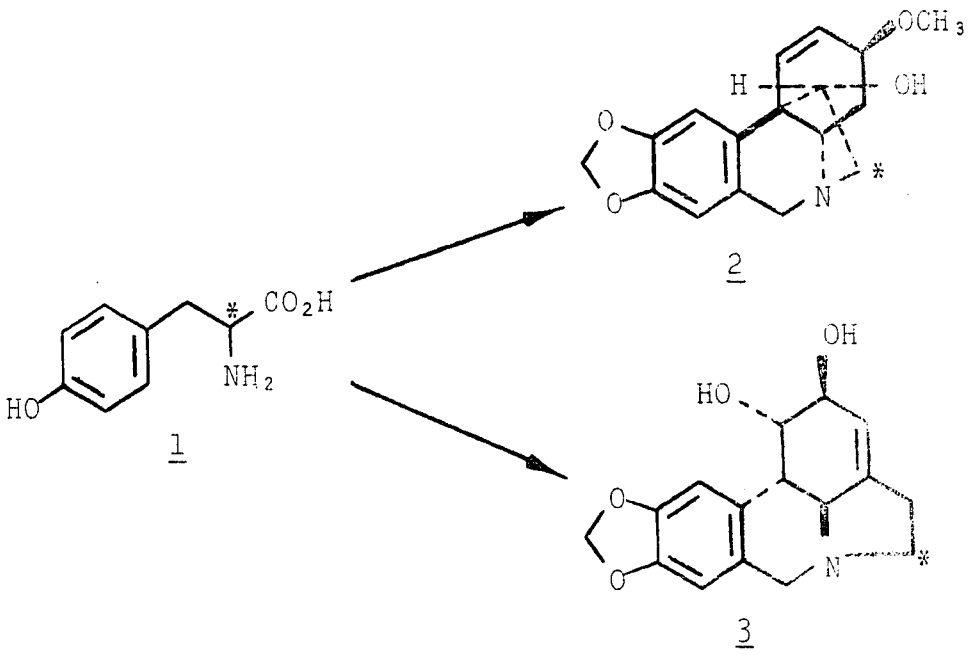
HISTORICAL

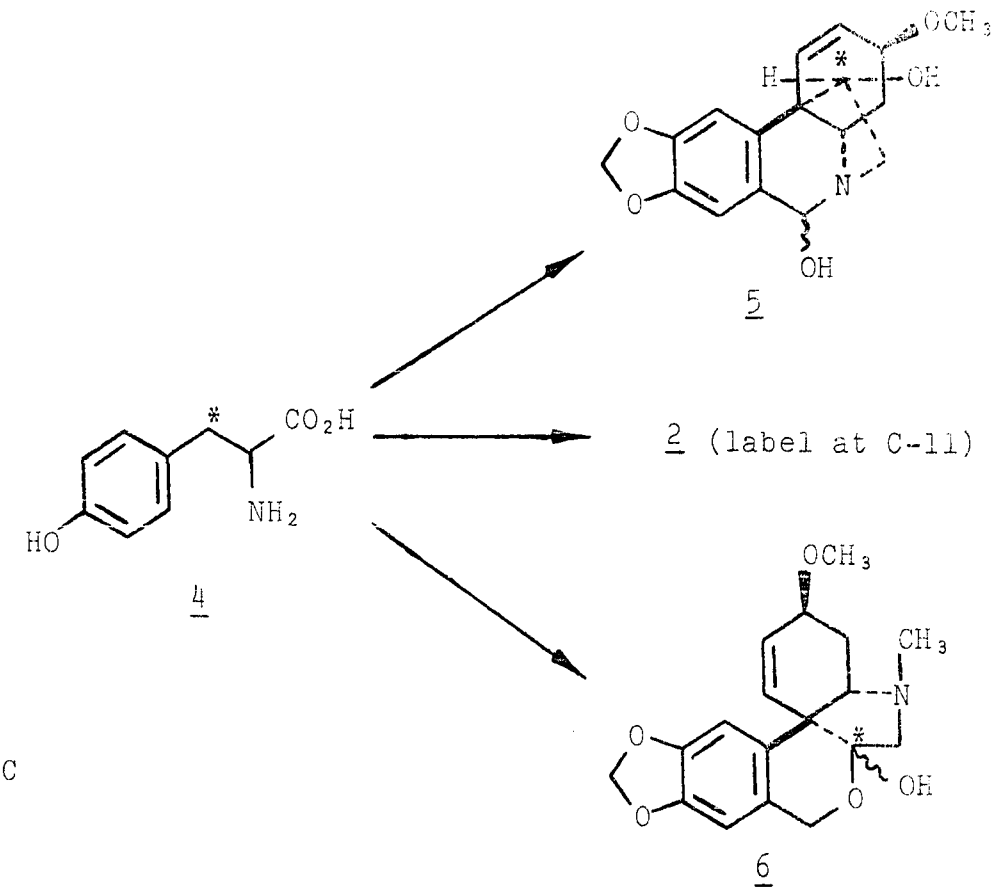
The Amaryllidaceae plant family has provided a great number of alkaloids of chemically diverse structure (1). This section will focus on the late stage biosynthetic transformations of the alkaloids.

Biosynthesis of the Amaryllidaceae Alkaloids

The major alkaloids of the Amaryllidaceae contain a fundamental ring system of fifteen carbon atoms which may be divided into a seven-carbon aromatic fragment, the C_6-C_1 unit and an eight-carbon hydroaromatic fragment, the C_6-C_2 unit. The first studies into the biosynthesis of the Amaryllidaceae alkaloids were concerned with the origins of these subunits (1). Logical amino acid precursors for the C_6-C_1 and C_6-C_2 units were phenylalanine and tyrosine.

Feeding experiments with ^{14}C-tyrosine have demonstrated conclusively that tyrosine was the precursor of the C_6-C_2 unit. When DL-[2-^{14}C]tyrosine (1) was administered to Narcissus "Twink", it was incorporated into haemanthamine (2) and lycorine (3) (2,3). DL-[3-^{14}C]Tyrosine (4) was incorporated into haemanthamine (2), haemanthidine (5) and tazettine (6) (4,5). Chemical degradation of the isolated alkaloids established that all of the radioactivity present was located in the indicated positions. These results


OCH3
H
OH
O
O
N
*
2
CO2H
*
NH2
HO
1
OH
HO
O
O
N
*
3


OCH3
H
*
OH
O
O
N
OH
5
CO2H
*
NH2
HO
4
2 (label at C-11)
OCH3
CH3
N
O
O
*
OH
O
6

* = ^{14}C

demonstrated that incorporation of tyrosine into the alkaloids was not preceded by total degradation of tyrosine in the plant and the absence of radioactivity in the C_6-C_1 unit eliminated tyrosine as a C_6-C_1 precursor.

Since no incorporation into the alkaloids occurred when DL-[1-^{14}C]tyrosine was administered to host plants (6), a logical explanation would be decarboxylation of the tyrosine to tyramine (14) followed by incorporation into the alkaloids. When feedings were carried out with [1-^{14}C]tyramine or [2-^{14}C]tyramine, the isolated alkaloids demonstrated efficient incorporation in the predicted positions (6,7,8).

When feeding experiments were carried out with DL-[2-^{14}C]phenylalanine, no incorporation into the alkaloids was observed (8). The carbon-14 label in DL-[3-^{14}C]-phenylalanine was incorporated into the C-6 position of haemanthamine (2) and the C-7 position of lycorine (3) in Narcissus incomparabilis (8,9). The conclusion drawn from these results was that phenylalanine (7) served only as a C_6-C_1 precursor. This conclusion was strengthened by the lack of incorporation of DL-[2-^{14}C]phenylalanine into the C and D rings of lycorine (3). Since the tyrosine isolated from the plant hosts was not radioactive, phenylalanine (7) was not converted to tyrosine before incorporation into the alkaloids. The generality of these findings was also demonstrated by other research groups (6,10,11).

The conversion of phenylalanine (7), a C_6-C_3 unit, into a C_6-C_1 unit involved the formal loss of a two-carbon fragment. One possible route for such a loss was suggested by the findings of Jordan and Hartman (12), who isolated an enzyme system capable of converting phenylalanine (7) into phenylserine. A second alternative was suggested by the discovery of phenylalanine deaminase in plant tissue (13,14). These discoveries plus the reports of the successful incorporation of protocatechuic aldehyde (8,9,10) into the alkaloids suggested the possibility of two degradation pathways for phenylalanine (7): (a) phenylalanine (7) —> phenylserine —> benzaldehyde —> protocatechuic aldehyde (11), or (b) phenylalanine (7) —> E-cinnamic acid (8) —> p-hydroxycinnamic acid (9) —> caffeic acid (10) or p-hydroxybenzaldehyde —> protocatechuic aldehyde (11).

Suhadolnik and coworkers (15) have presented evidence which eliminated path a and substantiated path b. When they fed [^{3}H]phenylserine, [7-^{14}C]benzaldehyde, and p-hydroxy[7-^{14}C]benzaldehyde to Narcissus pseudonarcissus, they found no appreciable incorporation into the alkaloids (15). When DL-[2-^{14}C]-p-hydroxyphenylserine was administered to Crinum erubescens, all of the radioactivity in the isolated alkaloids was located in the methoxyl and methylenedioxy functions. This indicated that degradation to a C_1 precursor preceded incorporation into the alkaloids (7).

Suhadolnik and coworkers (9,15,16,17) further demonstrated that efficient incorporation of E-[3-^{14}C]cinnamic acid, p-hydroxy[3-^{14}C]cinnamic acid, and [^{3}H]caffeic acid had occurred into the alkaloids. These results established path b (Figure 1) as the correct pathway and confirmed the earlier observations of Battersby and coworkers (6). As further proof of the correctness of path b, Suhadolnik and coworkers (15) reported the isolation of phenylalanine deaminase from the Amaryllidaceae.

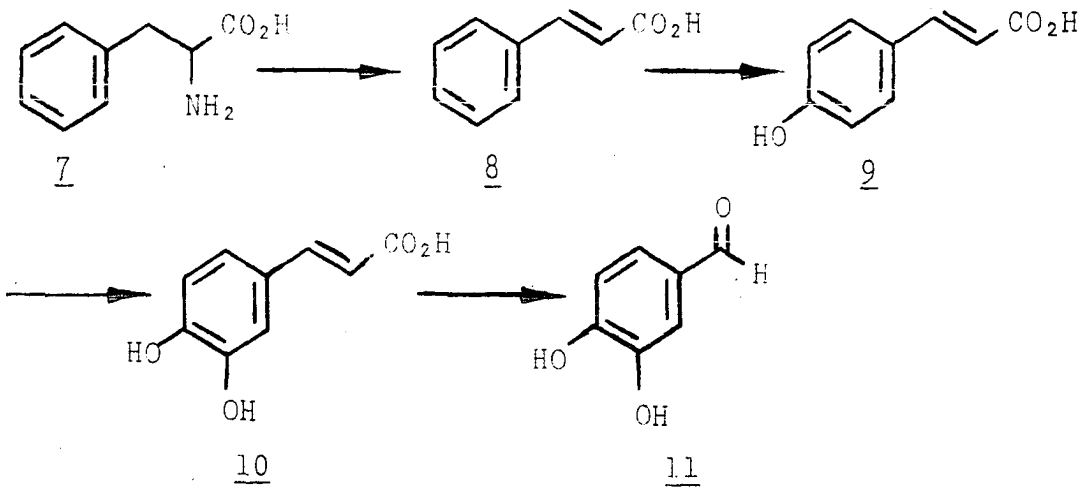
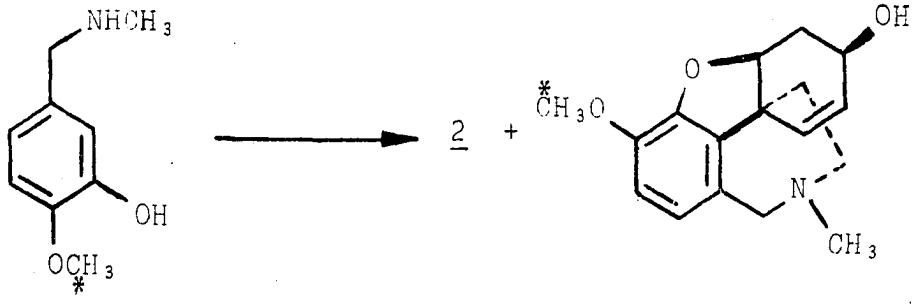


Figure 1. Conversion of phenylalanine (7) to protocatechuic aldehyde (11) via path b.

The possibility of other C_6-C_1 precursors was reported by Barton and coworkers (18), who found that N-[methyl-^{14}C]-3-hydroxy-4-[methoxy-^{14}C]benzylamine (12) was incorporated into galanthamine (13) and haemanthamine (2). Chemical degradations established that all of the radioactivity was located in the methoxyl group of galanthamine (13) and the methylenedioxy group of haemanthamine (2). They rationalized



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their results by suggesting that (12) was degraded to isovanillin or to 3-hydroxy-4-methoxybenzylamine and then incorporated into the alkaloids. Although previous feeding experiments with [^{3}H]isovanillin had found no evidence for its incorporation into the alkaloids (6), these results must be treated with caution because the poor solubility of isovanillin in water could result in difficulties in cellular transport.

Since tyramine (14) and protocatechuic aldehyde (11) were logical precursors of norbelladine (15), the proposed pathways from phenylalanine (7) and tyrosine to the C_6-C_1 and C_6-C_2 units were in agreement with the earlier postulate of Barton and Cohen (19) that norbelladine-type compounds could undergo oxidative phenyl-phenyl coupling in the plant to form the various alkaloids (Figure 2). Para-para coupling could provide crinine (16) (path a); ortho-para coupling could provide galanthamine (13) (path b); para-ortho coupling could provide lycorine (3) (path c). The subsequent isolation of belladine (15a) from Nerine bowdenii (20) and Amaryllis belladonna (21) coupled with the detection of O-methylnorbelladine (15b) by isotopic dilution in Narcissus pseudonarcissus L. var. "Twink" (6) further supported the postulate of Barton and Cohen.

The origins of the methylenedioxy group of the alkaloids has also been investigated. The intact incorporation of O-[methyl-^{14}C, 1'-^{14}C]norbelladine (15b) into haemanthamine (2) (18), O-[methyl-^{14}C, $^{3}H_4$]norbelladine into lycorine (3) (8), and the incorporation of [^{3}H]norpluviine (28) into lycorine (3) (6) eliminated an O-demethylation process and established that the carbon atom of the o-methoxy group becomes the carbon atom of the methylenedioxy group.

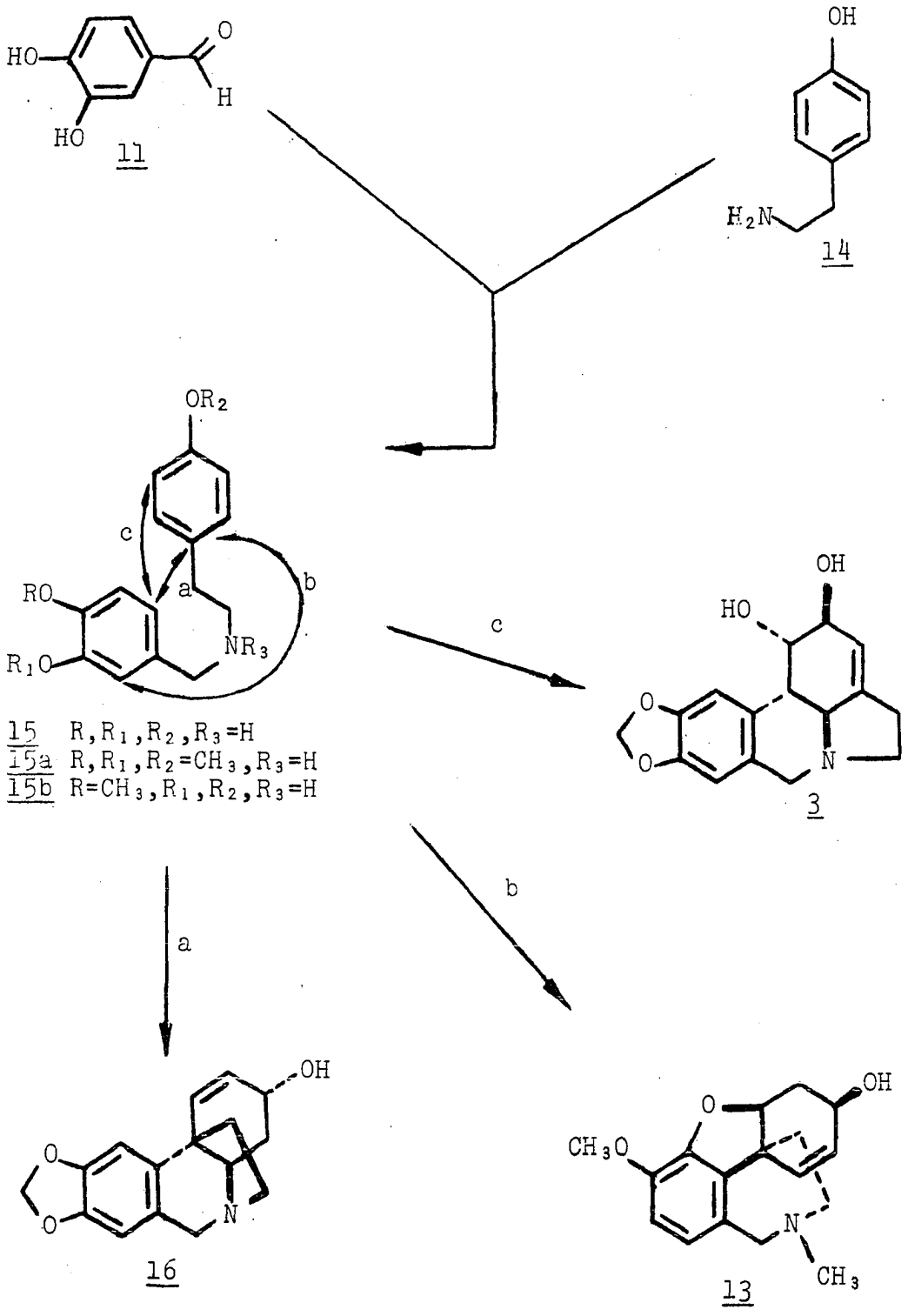
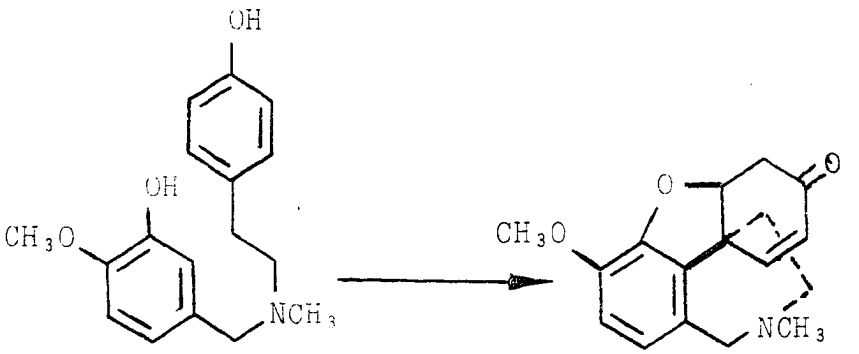


Figure 2. Biosynthetic pathways in the Amaryllidaceae.

Biogenetic Syntheses of the Amaryllidaceae Alkaloids

The phenyl-phenyl oxidative coupling theory provided several synthetic approaches to the Amaryllidaceae alkaloids (22). These approaches have in common the generation of a phenyl radical or a phenoxonium ion in a norbelladine-type system.

Barton and Kirby (23) obtained (±)-narwedine (18) in 1.4% yield when N,O-dimethylnorbelladine (17) was oxidized



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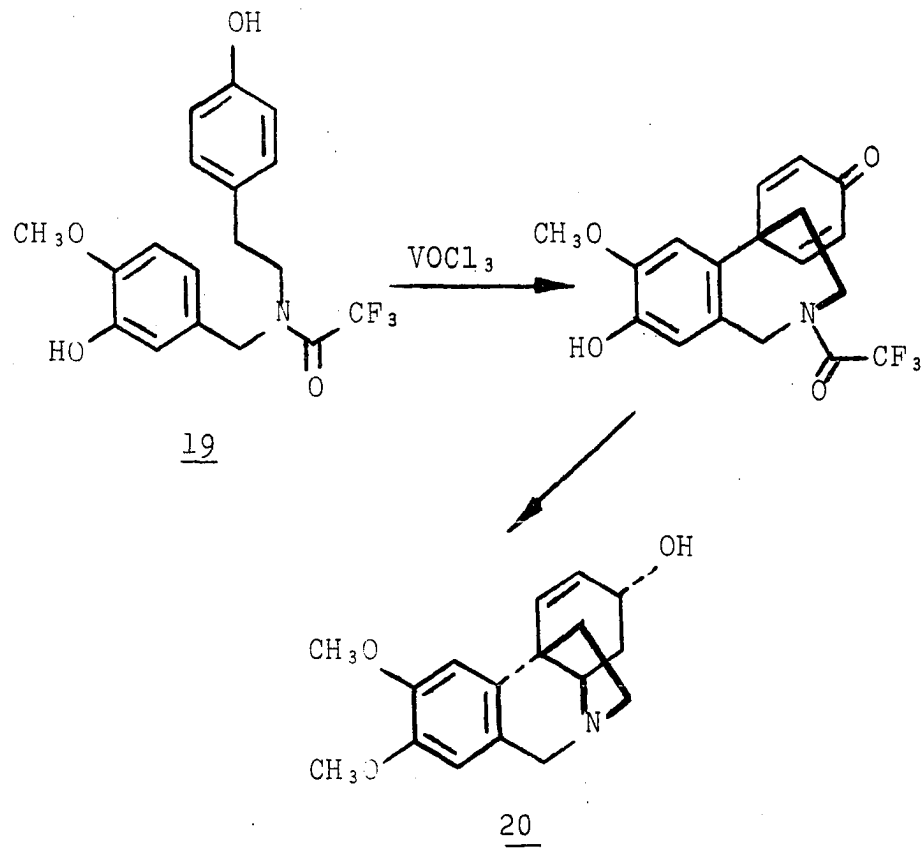
by alkaline potassium ferricyanide. Abramovitch and Takahashi (24), Franck and Lubs (25), and Franck and coworkers (26) reported that the yield could be improved markedly by protecting the amine function as an amide. Schwartz and Holton (27) obtained a 6.5% yield of (±)-maritidine (20) by oxidizing O-methylnorbelladine trifluoroacetamide (19) with vanadium oxytrichloride. Kametani and coworkers (28,29,30,31) have developed an improved synthesis of galanthamine (13) via the phenyl-phenyl oxidative coupling



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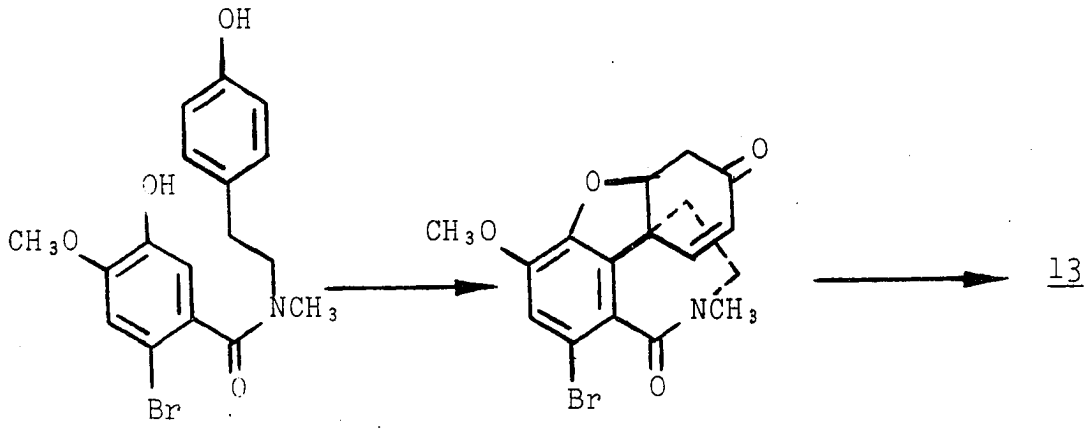
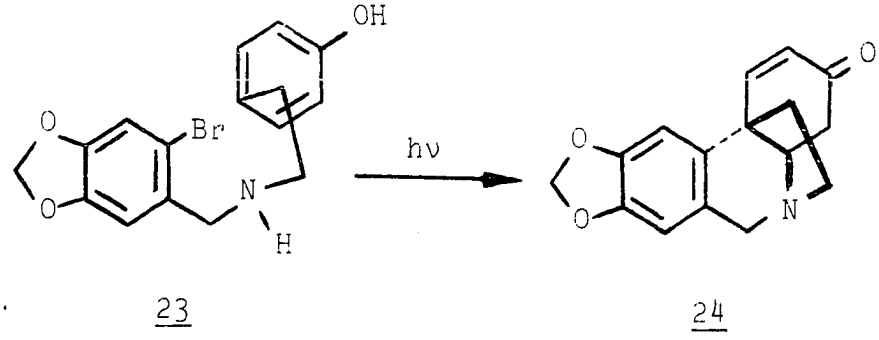
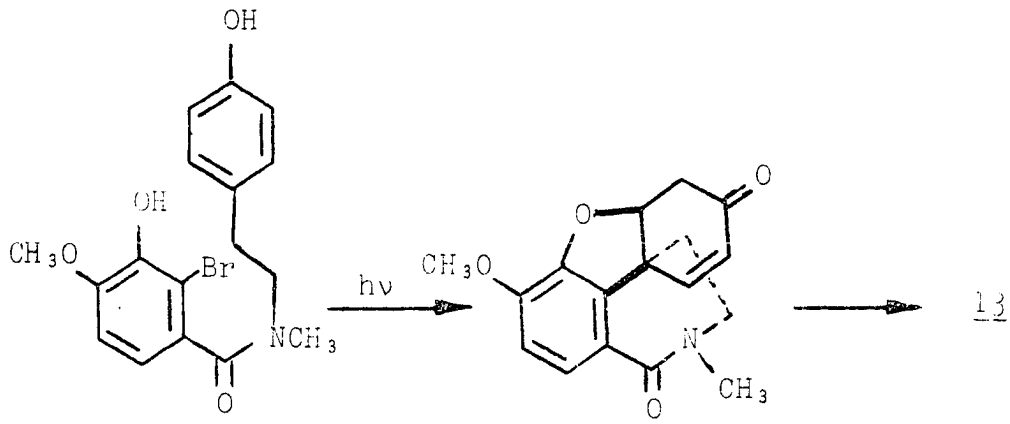
route by introducing a bromine atom into a position on the A ring to block the para-para coupling route and then incorporating the amide blocking group into the carbon skeleton of the O-methylnorbelladine derivative. When 2-bromo-5-hydroxy-N-(4-hydroxyphenethyl)-4-methoxy-N-methylbenzamide (21) was oxidized with potassium ferricyanide, the narwedine-type enone (22) was formed. Reduction with lithium aluminum hydride gave (±)-galanthamine (13) in an overall yield of 20%.



Kametani and coworkers (32,33,34) have also carried out the cyclization of norbelladine-type systems by generating a radical species via photolytic heterolysis of a carbon-bromine bond. When 2-bromo-N-(4-hydroxyphenethyl)-4,5-methylenedioxybenzylamine (23) was photolyzed, (±)-oxocrinine (24) was obtained in 5% yield (32). An analogous route was employed to obtain (±)-oxomaritidine (33).



Similarly, when 2-bromo-3-hydroxy-N-(4-hydroxyphenethyl)-4-methoxy-N-methylbenzamide (25) was irradiated and the resultant narwedine-type enone (26) was reduced with lithium aluminum hydride, (±)-galanthamine (13) was obtained in 0.6% yield (34).



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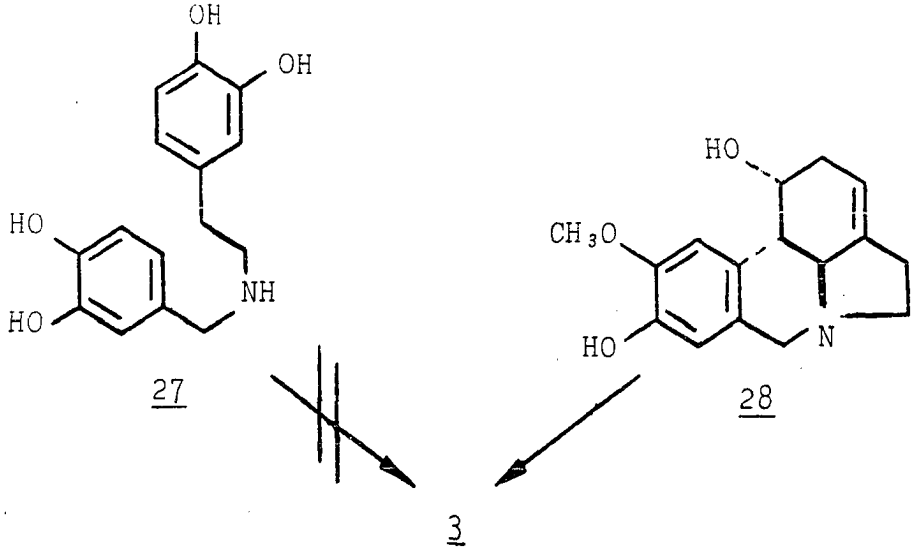
Two biogenetically patterned syntheses have recently been published which invoke the intermediacy of a phenoxonium ion. When N-(4-hydroxyphenethyl)-N-3,4-methylenedioxybenzyl trifluoroacetamide (27) was oxidized with thallium(III) trifluoroacetate, followed by hydrolysis of the amide, (±)-oxocrinine (24) was obtained in 15% yield (35). When the same oxidation was carried out at a platinum anode with 0.1 m fluoroboric acid as the electrolyte, (±)-oxocrinine (24) was obtained in a 59% yield (36). Both reactions are thought to involve two-electron oxidations of the phenol to a phenoxonium ion which can then carry out an electrophilic attack on the neighboring aromatic ring.

Late-stage Hydroxylations in the Amaryllidaceae Alkaloids

An area of present interest is the sequence and stereochemistry of hydroxylations in the late-stage modifications of many alkaloids including those of the Amaryllidaceae. With regard to the alkaloids of importance in this thesis, these hydroxylations can be broken down into three types; hydroxylations at C-2 in the lycorine alkaloids, hydroxylations at C-11 in the crinine alkaloids, and hydroxylations at the benzylic carbon atom alpha to the nitrogen in the alkaloids.

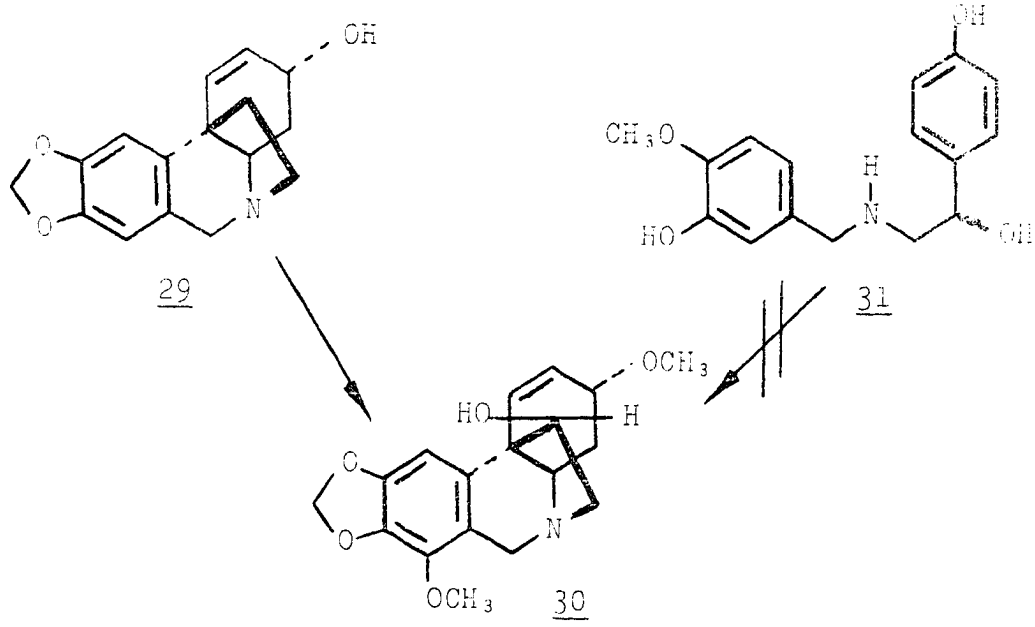
A great deal of evidence has been accumulated to show that these hydroxylations are late-stage transformations.

In the case of the C-2 hydroxylations in the lycorine alkaloids, it was initially shown that hydroxy[1'-^{14}C]norbelladine (27) was not incorporated into lycorine (3) (37). When [^{3}H]norpluviine (28) was fed, good incorporation into

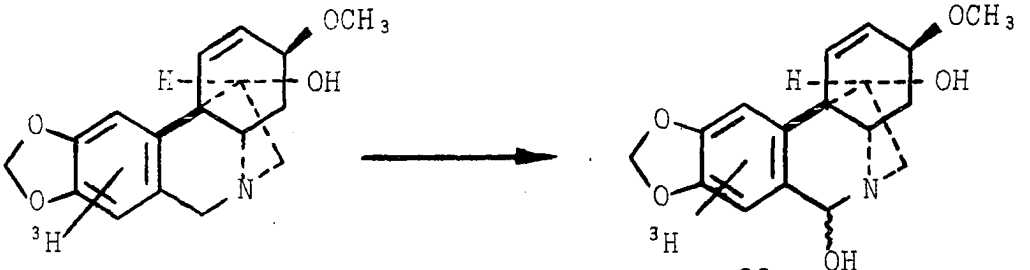


lycorine (3) was observed. This indicated that hydroxylation at C-2 occurs after the basic ring system is formed (6).

The conversion of [1,1'-^{14}C]norbelladine (15) (11) and [^{3}H]crinine (29) (38) into ambelline (30) in Nerine bowdenii established that the hydroxylations at C-11 in the crinine alkaloids occurred at a late stage in the biosynthetic pathway. This conclusion was further substantiated by the fact that octopamine and p-hydroxyphenylserine did not serve as precursors of either the C_6-C_1 or C_6-C_2 units but solely as precursors to the methoxyl and methylenedioxy units (7). Additional evidence was obtained by the lack of incorporation of 2-hydroxy-O-methyl[2-^{3}H,1'-^{14}C]norbelladine (31) into ambelline (30) in Crinum powellii (39). The incorporation of [^{3}H]vittatine, the enantiomer of crinine (29), into haemanthamine (2) (7) also established the late-stage timing of hydroxylation at C-11 in the crinine alkaloids.



Preliminary evidence for the late-stage hydroxylation at the benzylic carbon alpha to the nitrogen atom was established when it was found that [^{3}H]haemanthamine (32) was incorporated into haemanthidine (33) in Sprekelia formossissima (40).



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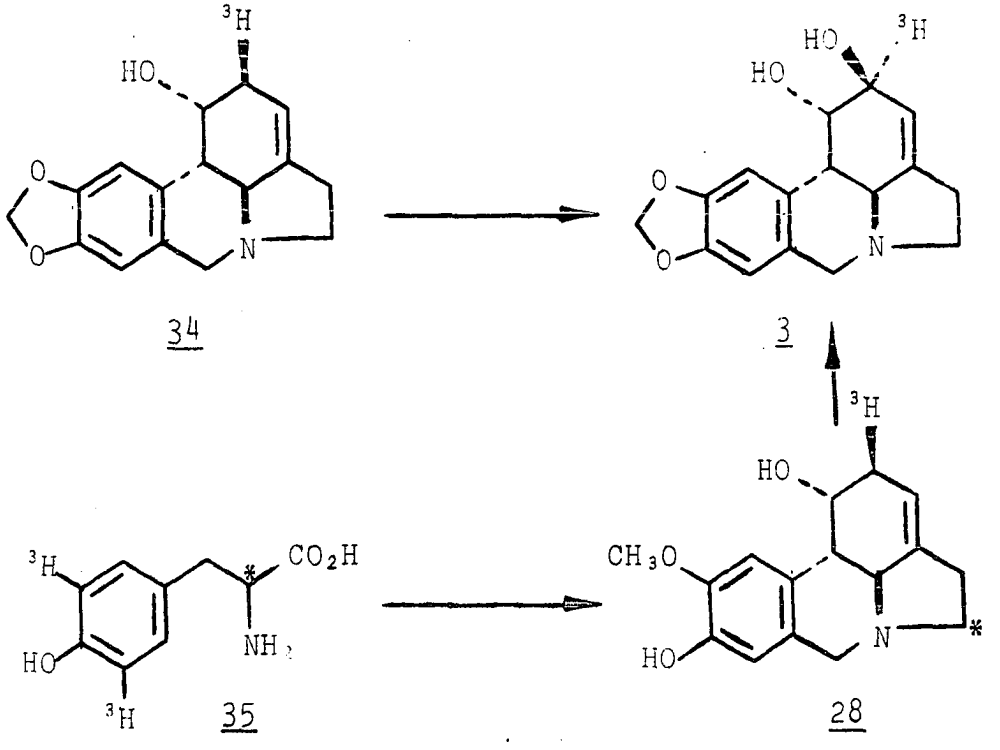
Wildman and Heimer (41,42) reported that hydroxylation at C-2 in lycorine (3) occurred with inversion of configuration. When [2β-^{3}H]caranine (34) was fed to Zephyranthes candida, the isolated lycorine (3) contained tritium at C-2.



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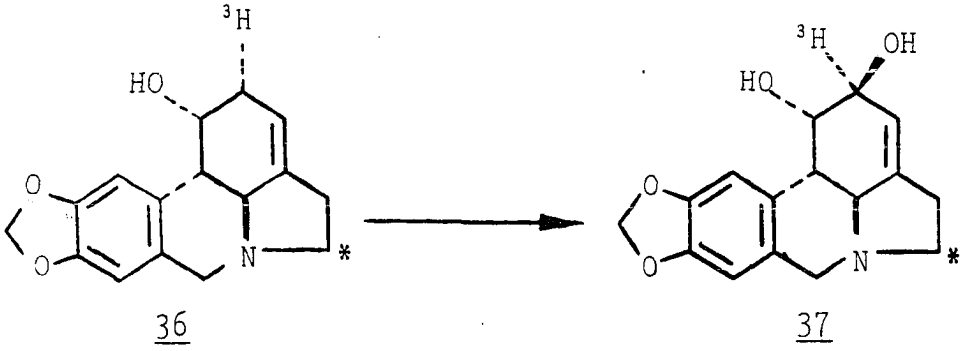
These results were substantiated by the findings of Bruce and Kirby (43,44). DL-[3,5-^{3}H,α-^{14}C]Tyrosine (35) was fed to "Twink" and "Deanna Durbin" daffodils. The tracer was incorporated into norpluviine (28) and lycorine (3), which were shown to have the same relative $^{3}H/^{14}C$ ratio (43). The tritium atom in norpluviine (28) was established to be in the β-configuration by chemical degradation (44). These results were the first experimental verification of an *in vivo* hydroxylation with inversion of configuration. Oxidation with inversion has been proposed as well in the hydroxylation at C-14 of the cardienolides (45) and bufadienolides (46).

In direct contrast, Fuganti and Mazza (47) fed [2α-^{3}H, 5-^{14}C]caranine (36) and [2β-^{3}H,5-^{14}C]norpluviine (37) to



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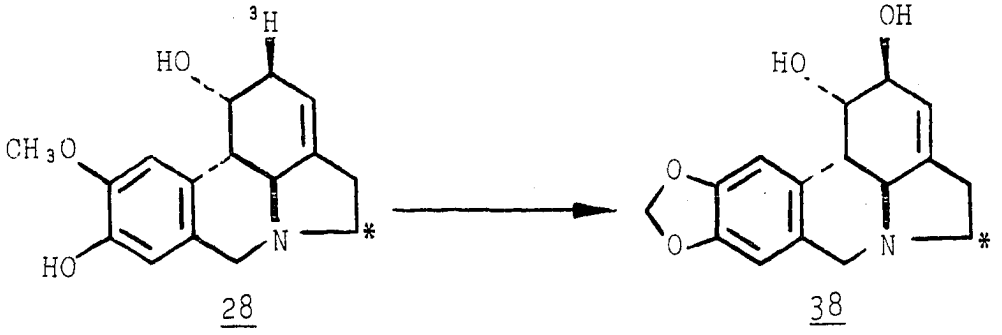

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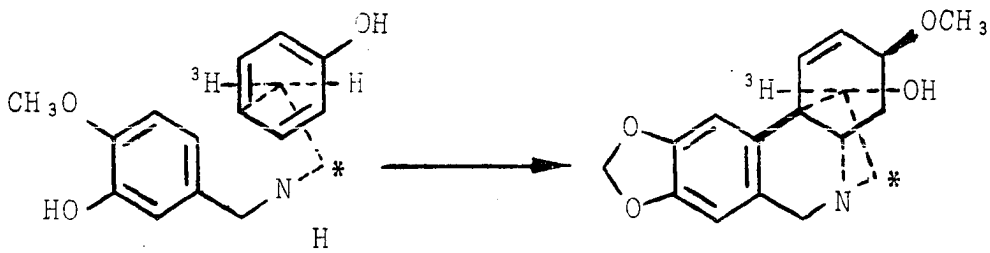
Clivia miniata. Their incorporation data for the isolated lycorine (37,38) suggested that the hydroxylation at C-2 in the lycorine alkaloids occurred via a retention of configuration mechanism in Clivia miniata.

The hydroxylation at C-11 of the crinine alkaloids has been independently investigated by two research groups. Battersby and coworkers (48) found that O-methyl[2S-^{3}H,1-^{14}C]-norbelladine (39) was converted into haemanthamine (40) with retention of tritium and O-methyl[2R-^{3}H,1-^{14}C]norbelladine (41) was incorporated into haemanthamine (42) with loss of tritium in Narcissus pseudonarcissus "King Alfred". When



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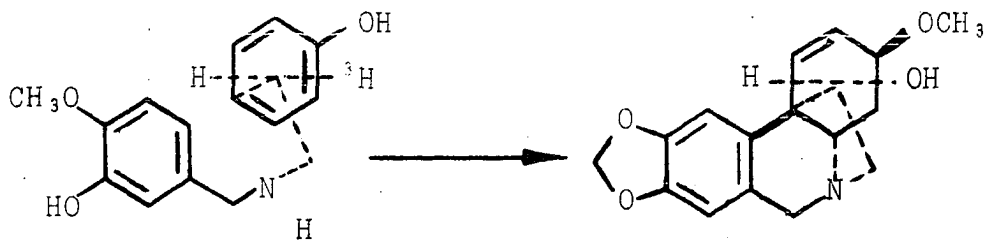

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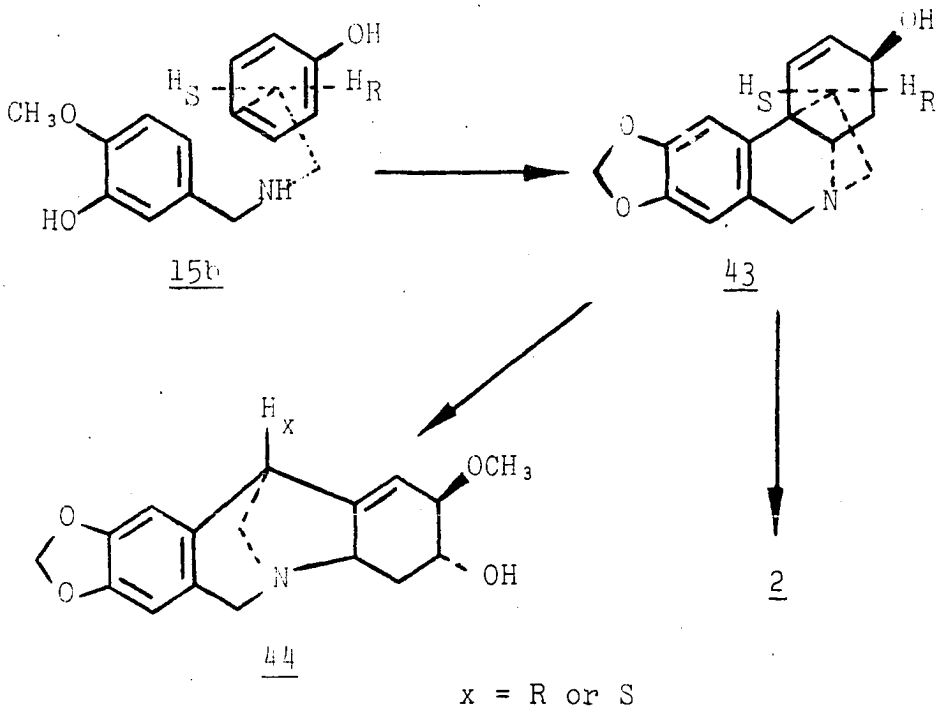
DL-[βR-^{3}H,α-^{14}C]tyrosine and DL-[βS-^{3}H,α-^{14}C]tyrosine were fed separately to "Texas" daffodils, Kirby and Michael (49, 50) isolated haemanthamine ((42) and (40) respectively) in which the C-11 hydroxylation had occurred via replacement of the pro-R hydrogen by a hydroxyl group with retention of configuration.

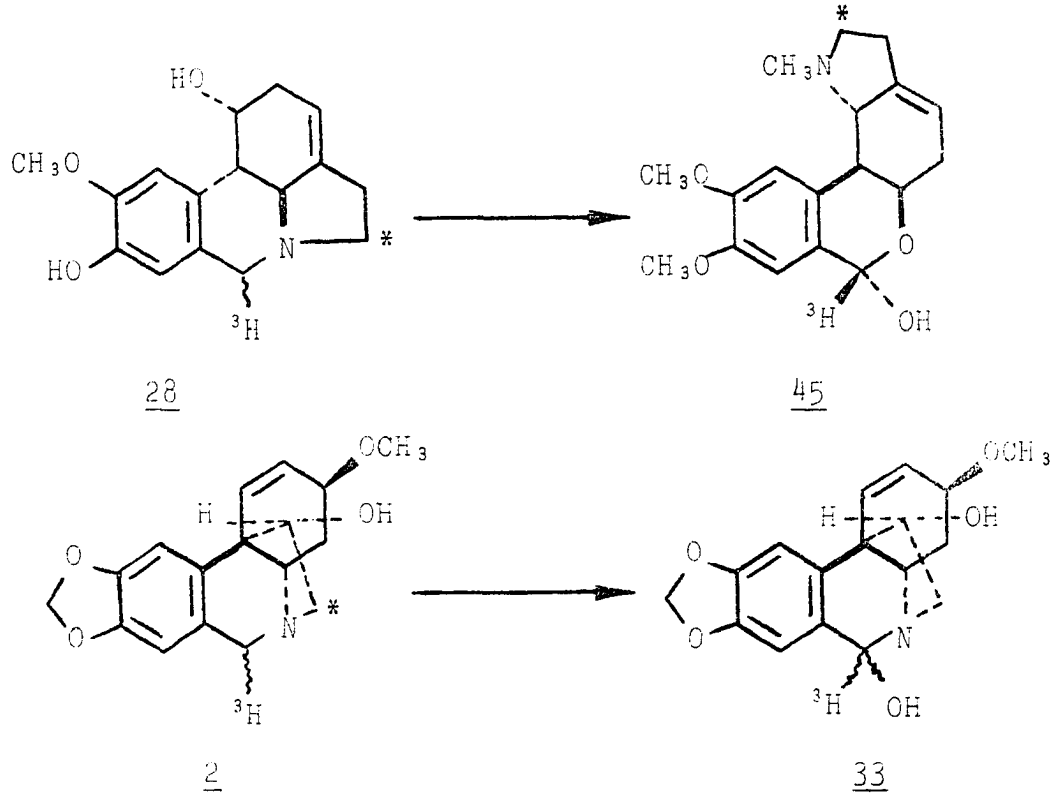
Fuganti and coworkers (51) have recently reported that (39) and (41) were incorporated into montanine (44) in Haemanthus coccineus with retention of the pro-R hydrogen and loss of the pro-S hydrogen. These results contradict the expected outcome based on the currently accepted view (1) of the biosynthesis of montanine (44). The montanine-type alkaloids were thought to be derived biosynthetically from O-methylnorbelladine (15b) by para-para coupling to form a haemanthamine-like intermediate which rearranged to the montanine skeleton. This biosynthetic route is based on the observation that [^{3}H]vittatine (43) was incorporated into haemanthamine (2) and montanine (44) (7) as well as the successful chemical conversion of 11-hydroxylated crinine derivatives into montanine alkaloids (52). In light of the findings observed in the biosynthesis of haemanthamine (2) (48,49,50), one would predict that H_S would be retained in montanine (44). The results of Fuganti and Mazza may require a re-evaluation of the proposed biosynthetic route to montanine (44) or the postulation of an alternate oxidation



mechanism for the formation of haemanthamine (2) in Haemanthus coccineus.

Fuganti and Mazza have carried out the only studies of hydroxylation at the benzylic position alpha to the nitrogen atom of the Amaryllidaceae alkaloids. In daffodils, [7-^{3}H]-protocatechuic aldehyde (11) was incorporated into norpluviine (28) and haemanthamine (2) via O-methylnorbelladine (15b) without loss of tritium. The isolated (28) and (2) were mixed with identical carbon-14 labeled material obtained by feeding O-methyl[1-^{14}C]norbelladine to daffodils. The doubly-labeled (28) and (2) were then refed to the previous

plant hosts. The doubly-labelled (28) was incorporated into lycorenine (45) and the double-labeled (2) was incorporated into haemanthidine (33) without loss of tritium (53).



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These results suggested that the hydrogen introduced at C-1' in the biosynthesis of O-methylnorbelladine (15b) from protocatechuic aldehyde (11) is removed by hydroxylation in the biosynthesis of lycorenine (45) and haemanthidine (33). When O-methyl[1'R-^{3}H,1-^{14}C]norbelladine was fed to daffodils, it was incorporated into norpluviine (28) and then into lycorenine (45). The conversion of (28) into (41) proceeded with complete loss of the pro-R hydrogen (54). The combined

results of these feeding experiments suggested that the hydrogen introduced in the biosynthesis of O-methyl-norbelladine (15b) entered from the re-face of the molecule.

RESULTS AND DISCUSSION

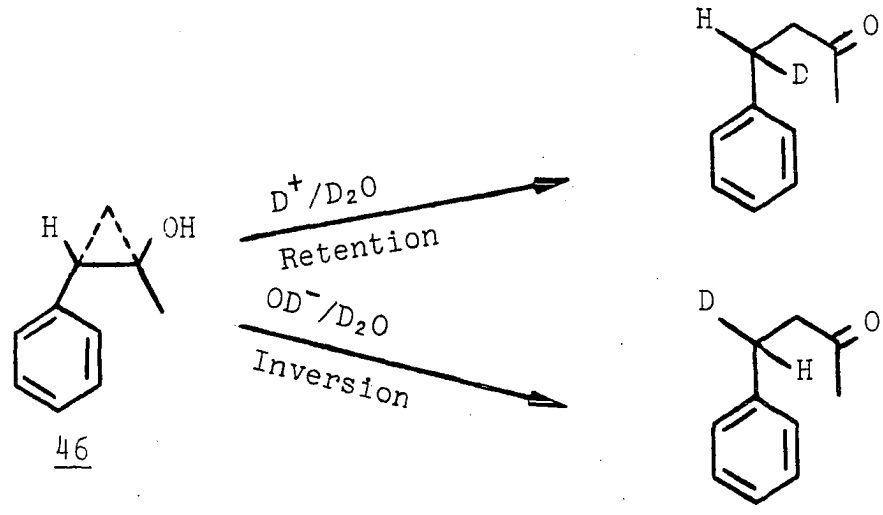
This section will discuss attempted synthetic preparations of (R)- or (S)-O-methyl[2-^{3}H]norbelladine, the synthetic preparation of O-methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79), 3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (92), and N-methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (93), their degradations, and the results of the biosynthetic feeding experiments.

Synthetic Investigations

Attempted syntheses of (R)- or (S)-O-methyl[2-^{3}H]norbelladine

An investigation of the hydroxylation process at C-11 of the crinine ring system would require the synthesis of (R)- or (S)-[β-^{3}H]tyramine (56), which could then be condensed with isovanillin to yield the desired (R)- or (S)-O-methyl-[2-^{3}H]norbelladine.

The original approach to the desired tyramine (56) was suggested by the work of Depuy and coworkers (55). Their investigations of the stereochemistry of the acid- and base-catalyzed ring openings of (-)-Z-1-methyl-2-phenylcyclopropanol (46) demonstrated that ring cleavage occurred with retention of configuration in an acid medium and inversion of configuration in a basic medium at the benzylic position.



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An approach directly paralleling the synthetic scheme of Depuy and coworkers (55) is outlined in Figure 3. There are a number of advantages inherent in this approach. The most important advantage is that the acid (47) is of known absolute configuration thus permitting the assignment of stereochemistry to the product. The acid (47) is also readily available in an optically active form *via* the method of Depuy and coworkers (55).

The successful completion of the synthesis of (R)- or (S)-[β-^{3}H]tyramine (56) would seem to require only the introduction of an oxygen functionality at the *para*-position of the phenyl ring. The introduction of a methoxyl group *via* the introduction of a *para*-nitro function, reduction of the nitro group to an amine, diazotization, hydrolysis of the diazonium salt, and methylation of the phenol proceeded

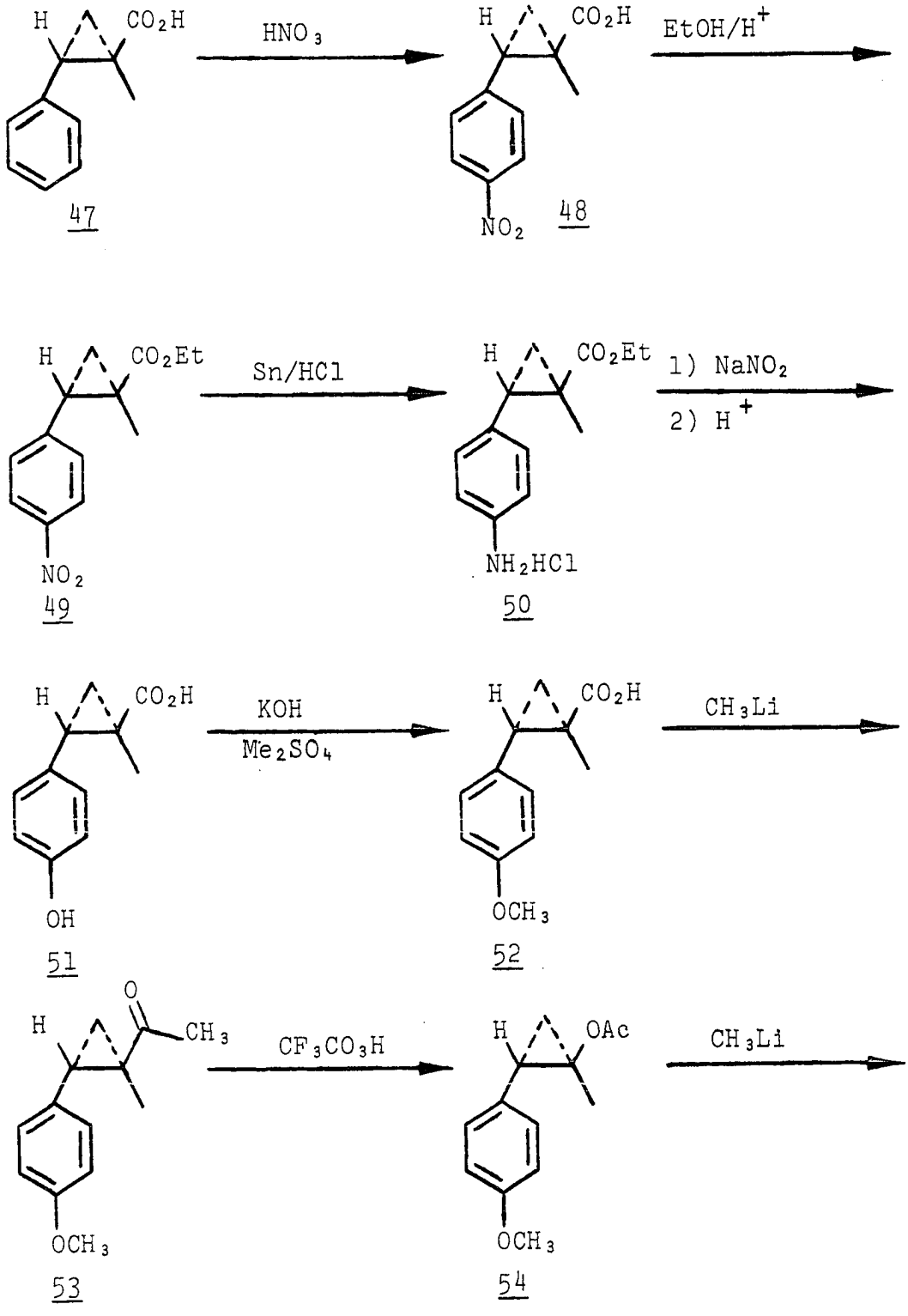
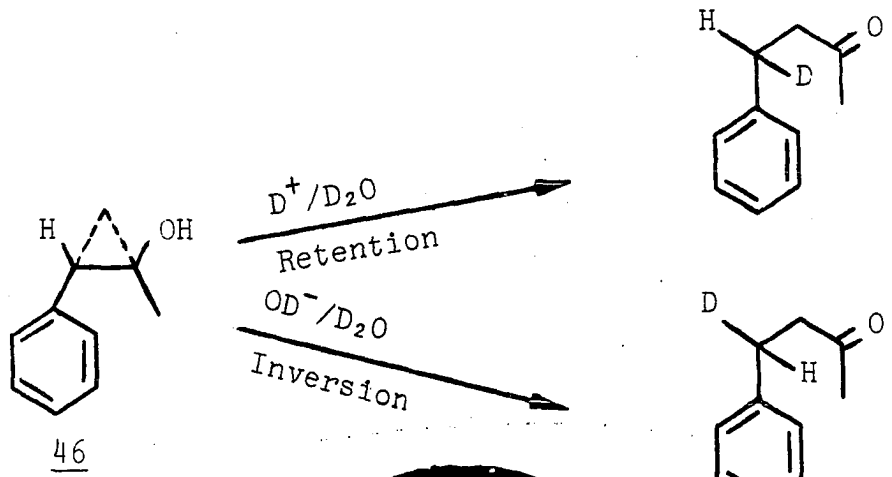


Figure 3. Attempted synthesis of (R)- or (S)-[2-^{3}H]tyramine *via* a cyclopropanol opening.



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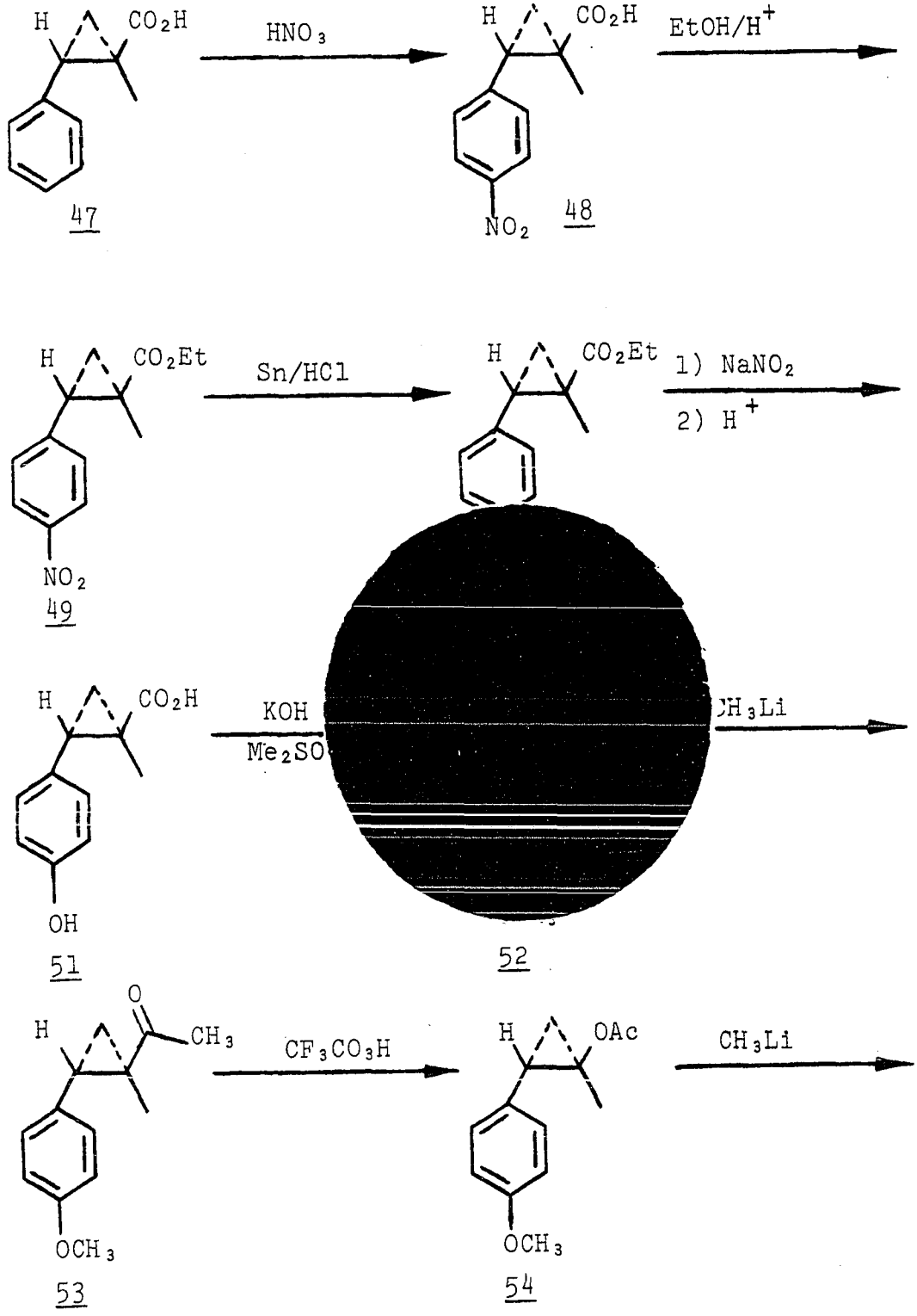
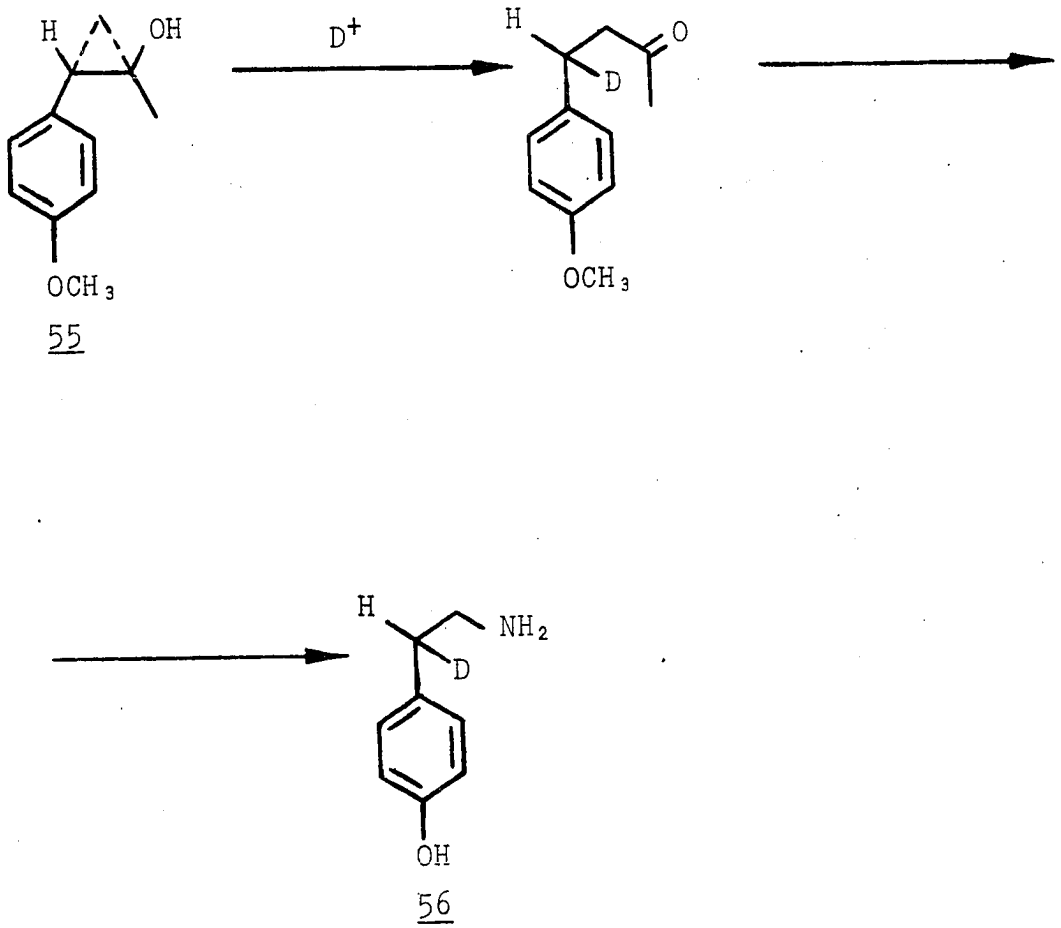


Figure 3. Attempted synthesis of (R)- or (S)-[2-^{3}H]tyramine *via* a cyclopropanol opening.



55

56

Figure 3 (Continued)

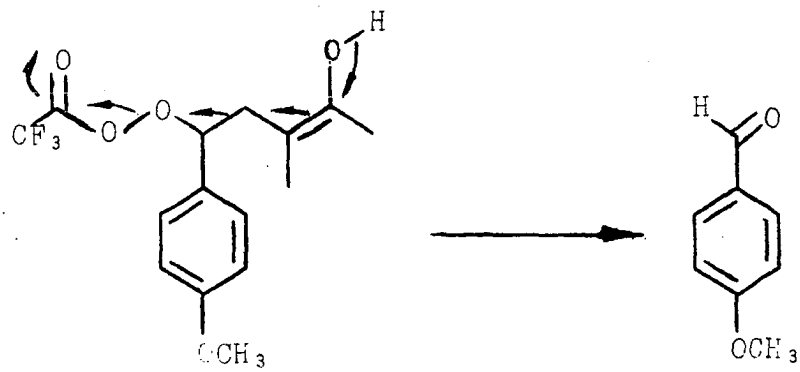
smoothly. The synthesis was greatly facilitated by the crystallization of only the p-nitro acid (48) from cold concentrated nitric acid during the nitration reaction.

Once the synthesis of the p-methoxyl acid (52) was accomplished, its conversion to the cyclopropyl methyl ketone (53) proceeded in a quantitative manner upon treatment with methyl lithium.

The only remaining key step at that point was the successful completion of the Baeyer-Villiger rearrangement of (53) to the cyclopropyl acetate (54). When the oxidation of the cyclopropyl methyl ketone (53) was attempted with trifluoroperacetic acid, a multi-component mixture was obtained. The crude mixture was divided into two fractions by column chromatography on silica gel. The less polar fraction was identified as anisaldehyde (53b). The more polar fraction was base soluble and gave a positive test for phenols when tested with ferric chloride. No evidence for the existence of the desired cyclopropyl acetate (54) could be found. The same results were observed when permaleic acid was substituted for trifluoroperacetic acid.

The formation of small amounts of phenols when an activated benzene ring is present under the reaction conditions employed for the Baeyer-Villiger rearrangement with a strong peracid is a well documented occurrence (56).

The production of anisaldehyde (53b) could be rationalized in the following manner. Protonation of the ketone (53) followed by nucleophilic attack by trifluoroperacetic acid at the benzylic position could lead to (53a). Loss of a proton could be followed by breakage of a carbon-carbon bond and elimination of trifluoroacetic acid with the resultant formation of anisaldehyde (53b).



53a 53b

Weaker peracids, such as m-chloroperbenzoic, peracetic, and perbenzoic, had no effect on the cyclopropyl methyl ketone (53) even after prolonged reaction times. In every instance, the ketone (53) was recovered quantitatively from the reaction mixture.

A second approach to the synthesis of (R)- or (S)-[β-^{3}H]tryamine (56) through a mandelic acid system was suggested by the work of Green (57) and by additional findings of Depuy and coworkers (55). This approach is outlined in

Figure 4. p-Benzyloxymandelic acid (58) could be obtained in good yield from p-benzyloxybenzaldehyde (57) by the method of Compere (58). The acid (58) could be cleanly resolved into the (-)-acid (58) with cinchonine. The reduction of the ester (59) with lithium aluminum hydride to the diol, protection of the 1-alcohol function as a trityl ether, conversion of the 2-alcohol function to the chloride with thionyl chloride, and displacement of the chloride with lithium aluminum hydride proceeded smoothly. The selective cleavage of the trityl ether function was accomplished by passing (62) through a column of Woelm silica gel (act. 1). The resultant alcohol (63) could then be converted to the desired tyramine (56).

When optically active material was carried through the sequence, racemization was observed during the conversion of the optically active diol (60) ($[\alpha]_D^{25} = -27.2°$) to the ether (61). The isolated 2-hydroxy-2-(p-benzyloxyphenyl)-ethyl trityl ether demonstrated no measurable optical rotation and the recovered diol (60) exhibited a small rotation, $[\alpha]_D^{25} = -6.08°$. A series of control experiments was initiated in an effort to establish the cause of the observed racemization. No evidence of racemization was observed in the absence of trityl chloride. When the conversion of the optically active diol (60) to the ether (61) was repeated, racemization was again observed.

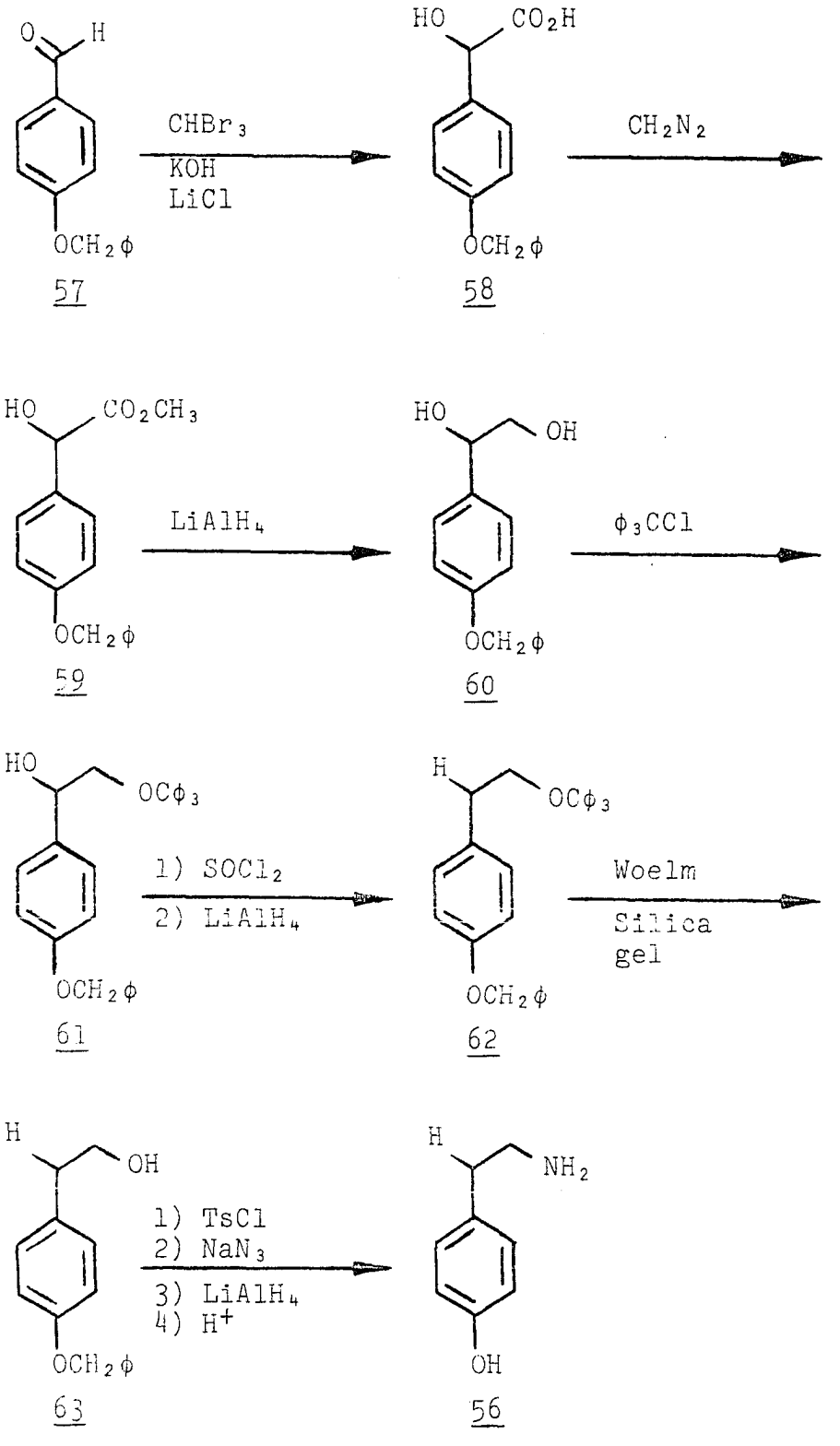


Figure 4. Attempted synthesis of (R)- or (S)-[β-^{3}H]tyramine (56) from p-benzyloxymandelic acid (58).

The publication of the results of Battersby and co-workers (48) and of Kirby and Michael (49,50) as well as the difficulties encountered in carrying out the synthetic sequences prompted the cancellation of these endeavors.

Synthesis and degradation of O-methyl[1'R-^{3}H,1-^{14}C]-norbelladine hydrochloride

An investigation of the hydroxylation process at the benzylic position alpha to the tertiary nitrogen atom in the Amaryllidaceae alkaloids would require the synthesis of an optically active O-methyl[1'-^{3}H,1-^{14}C]norbelladine hydrochloride.

The synthesis of O-methyl[1'R-^{3}H]norbelladine hydrochloride (71) is outlined in Figure 5. The key point of this synthesis was the use of the enzyme, equine liver alcohol dehydrogenase (LADH), and nicotinamide-adenine dinucleotide (NAD) to introduce the necessary stereochemistry. Reduction of the labeled aldehyde (66) with LADH gave 3-benzyloxy-4-methoxy[7S-^{3}H]benzyl alcohol (67) in good yield.

It was found during the labeling of the morpholino acetonitrile (65), that the neutralization of the reaction mixture with exactly one equivalent of thionyl chloride must be performed quickly and the reaction mixture must then be poured immediately into water. If more than one equivalent of thionyl chloride was used or the reaction mixture was

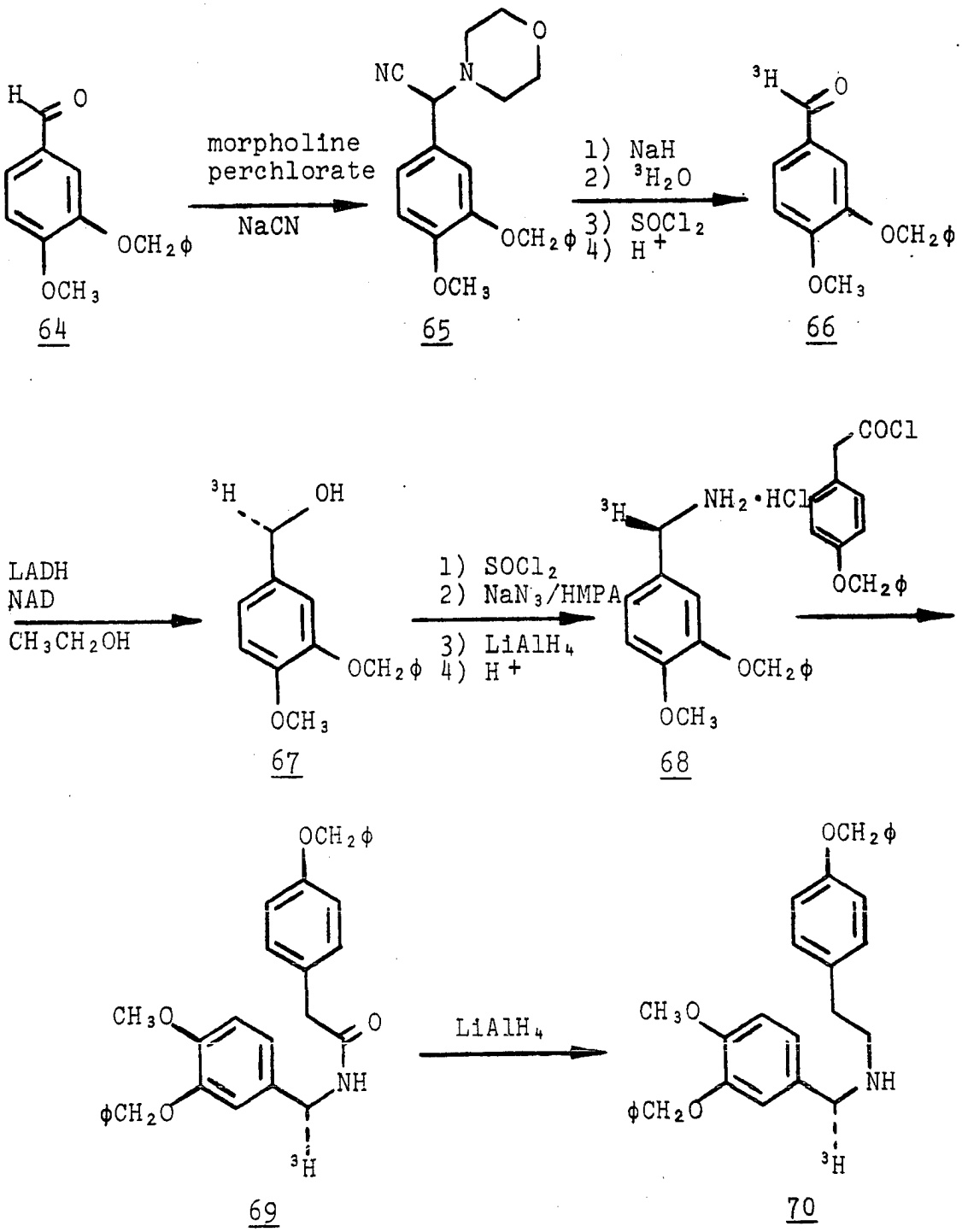
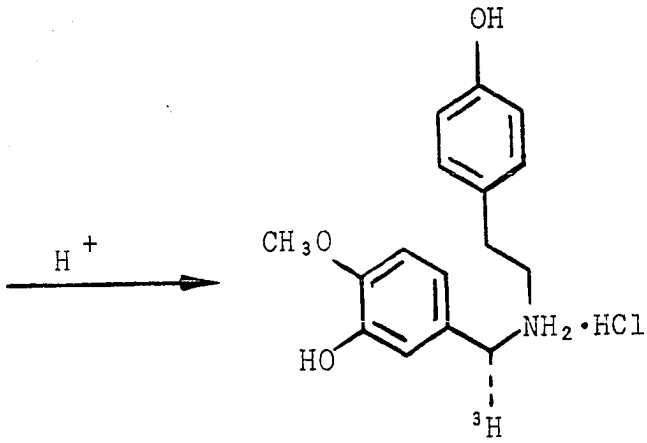


Figure 5. Synthesis of O-methyl[1'R-^{3}H]norbelladine hydrochloride.



71

Figure 5 (Continued)

not quickly diluted with water after neutralization, some tritium was exchanged into the benzene ring.

Once the 3-benzyloxy-4-methoxy[7R-^{3}H]benzylamine hydrochloride (68) was formed, no further chemical operations occurred at the asymmetric center and a degradation of (68) could establish the absolute configuration, the optical purity, and position of the tritium label. The amine hydrochloride (68) was degraded according to the scheme outlined in Figure 6. A small portion of (68) was diluted with non-radioactive material and carried through the outlined sequence of reactions. The conversion of the (R)-amine hydrochloride (68) to the (S)-alcohol (75) by this reaction sequence was assumed to be nearly 100% stereospecific based on the data reported for similar types of compounds (59,60). Snyder and Brewster (59) reported that the conversion of (+)-α-phenethyl amine to (-)-α-phenethyl acetate by the same series of reactions outlined in Figure 6 occurred with 98-100% inversion of configuration. Streitwieser and Wolfe (60) found essentially complete inversion of configuration during the conversion of (+)-[7-^{2}H]benzylamine to (-)-[7-^{2}H]benzyl alcohol by this route (Figure 6). These findings suggested that less than 5% racemization would occur during the conversion of the (R)-amine hydrochloride (68) to the (S)-alcohol (75).

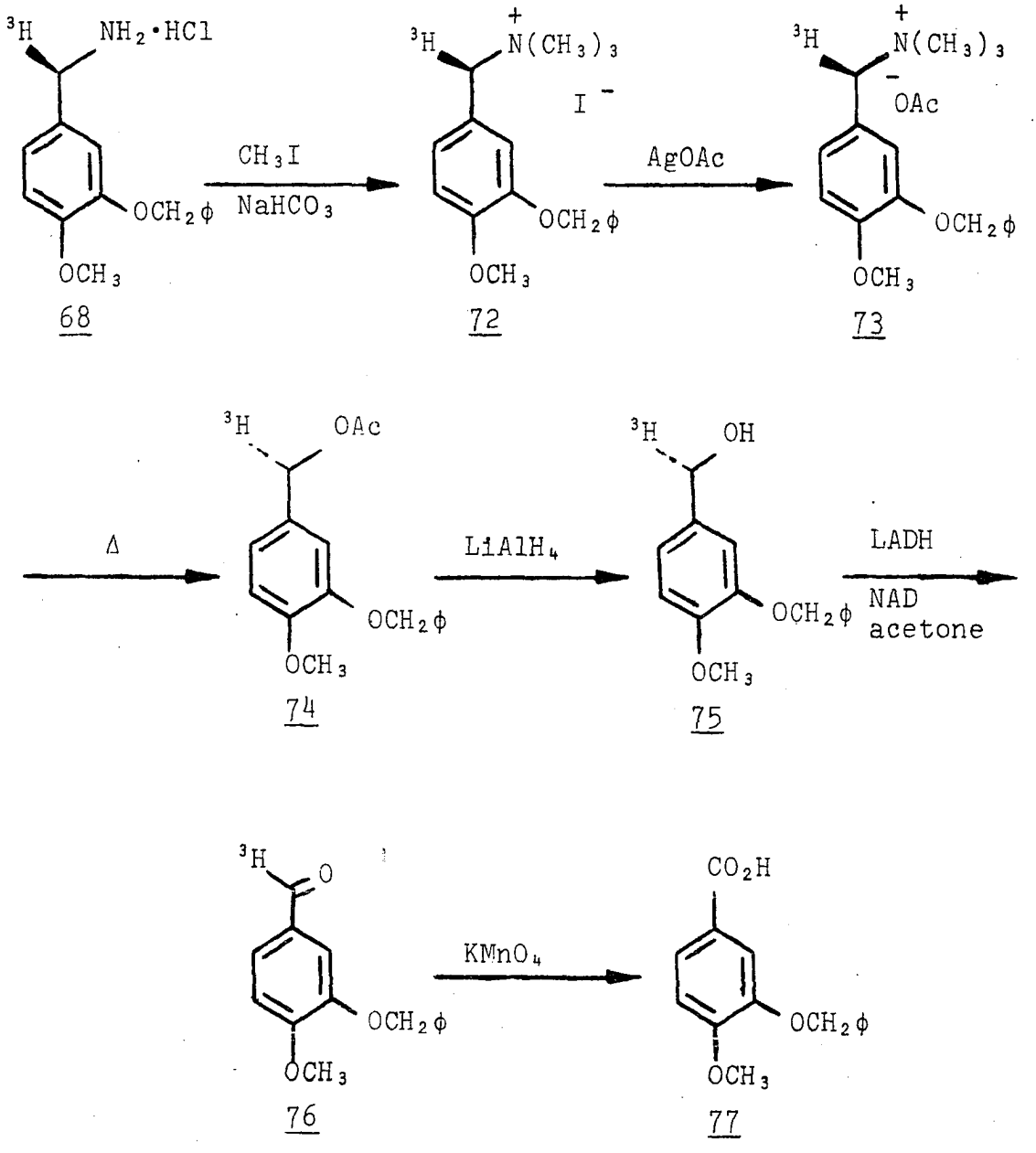


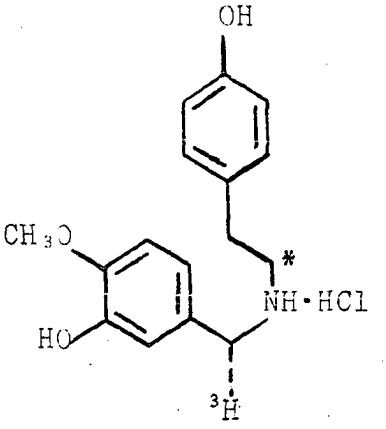
Figure 6. Degradation of 3-benzyloxy-4-methoxy[7R-^{3}H]-benzylamine hydrochloride.

The oxidation of the (S)-alcohol (75) to the aldehyde (76) with LADH, NAD, and acetone proceeded smoothly. In an effort to insure complete oxidation and minimize any possible error due to isotope effects, additional portions of LADH were added at intervals of two to three hours. Since the isotope effects, k_H/k_T, observed for LADH oxidations of an alcohol to an aldehyde were in the range of 1.3 to 1.8 at 24° and quickly approached 1.0 as the temperature approached 37° (61), the error attributed to an isotope effect was considered negligible. Since this oxidation involved the stereospecific removal of the pro-R hydrogen, the ratio of the specific activity of the aldehyde (76) (^3H - 3.79×10^8 dpm/mmol) to that of the (S)-alcohol (75) (^3H - 5.54×10^8 dpm/mmol) times one hundred was equal to the percentage of the tritium which occupied the pro-S position in the (S)-alcohol (75). This enzyme-catalyzed oxidation established that the labeled alcohol contained 68% of the (S)-alcohol (75) and therefore the labeled amine hydrochloride contained 68±5% of the (R)-amine hydrochloride (68). This value corresponded within experimental error to the value of 75±10% determined by Fuganti and Mazza (54), who synthesized the (R)-amine hydrochloride (68) by the method illustrated in Figure 5.

Oxidation of the aldehyde (76) (^3H - 3.79 x 10^6 dpm/mmol) to the corresponding benzoic acid (77) (^3H - 1.82 x 10^4 dpm/mmol) with potassium permanganate established that essentially all of the tritium label was at the benzylic position.

O-methyl[1-^{14}C]norbelladine hydrochloride (78) was synthesized by the method of Barton and coworkers (18) which is outlined in Figure 7. The position of the carbon-14 label has been established by Chan (39).

Mixing of O-methyl[1'R-^3H]norbelladine hydrochloride (71) and O-methyl[1-^{14}C]norbelladine hydrochloride (78) gave the doubly-labeled O-methyl[1'R-^3H,1-^{14}C]norbelladine hydrochloride (79).



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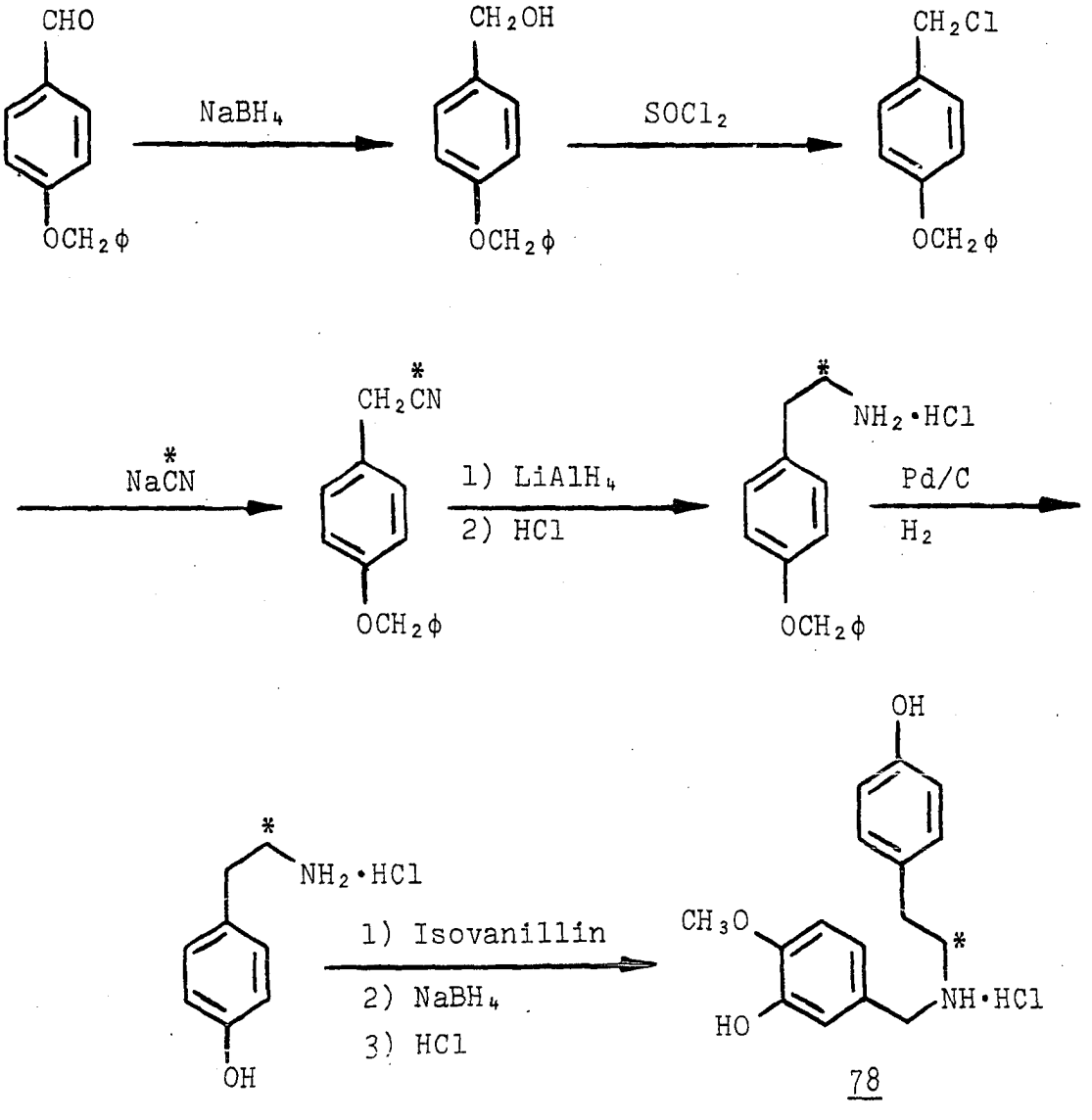
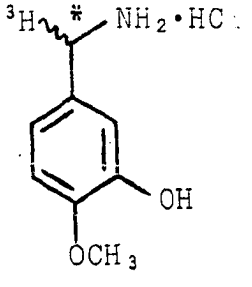


Figure 7. Synthesis of O-methyl[1-^{14}C]norbelladine hydrochloride.

Synthesis and degradation of 3-hydroxy-4-methoxy[7-^{3}H,^{14}C]-benzylamine hydrochloride and N-methyl-3-hydroxy-4-methoxy-[7-^{3}H,^{14}C]benzylamine hydrochloride

The syntheses of 3-hydroxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (82) and its N-methyl analog (84) are outlined in Figure 8. The benzyloxy[7-^{3}H]isovanillin (66) was prepared in separate lots for each synthesis.

The syntheses of 3-hydroxy-4-methoxy[7-^{14}C]benzylamine hydrochloride (88) and N-methyl-3-hydroxy-4-methoxy[7-^{14}C]-benzylamine hydrochloride (91) are outlined in Figure 9. The acid (85) was prepared by the method of Chan (39). Mixing of (82) and (88) gave the doubly-labeled 3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (92). Mixing of (84) and (91) gave the doubly-labeled N-methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (93).



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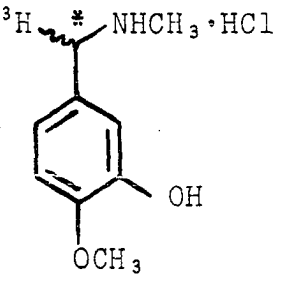

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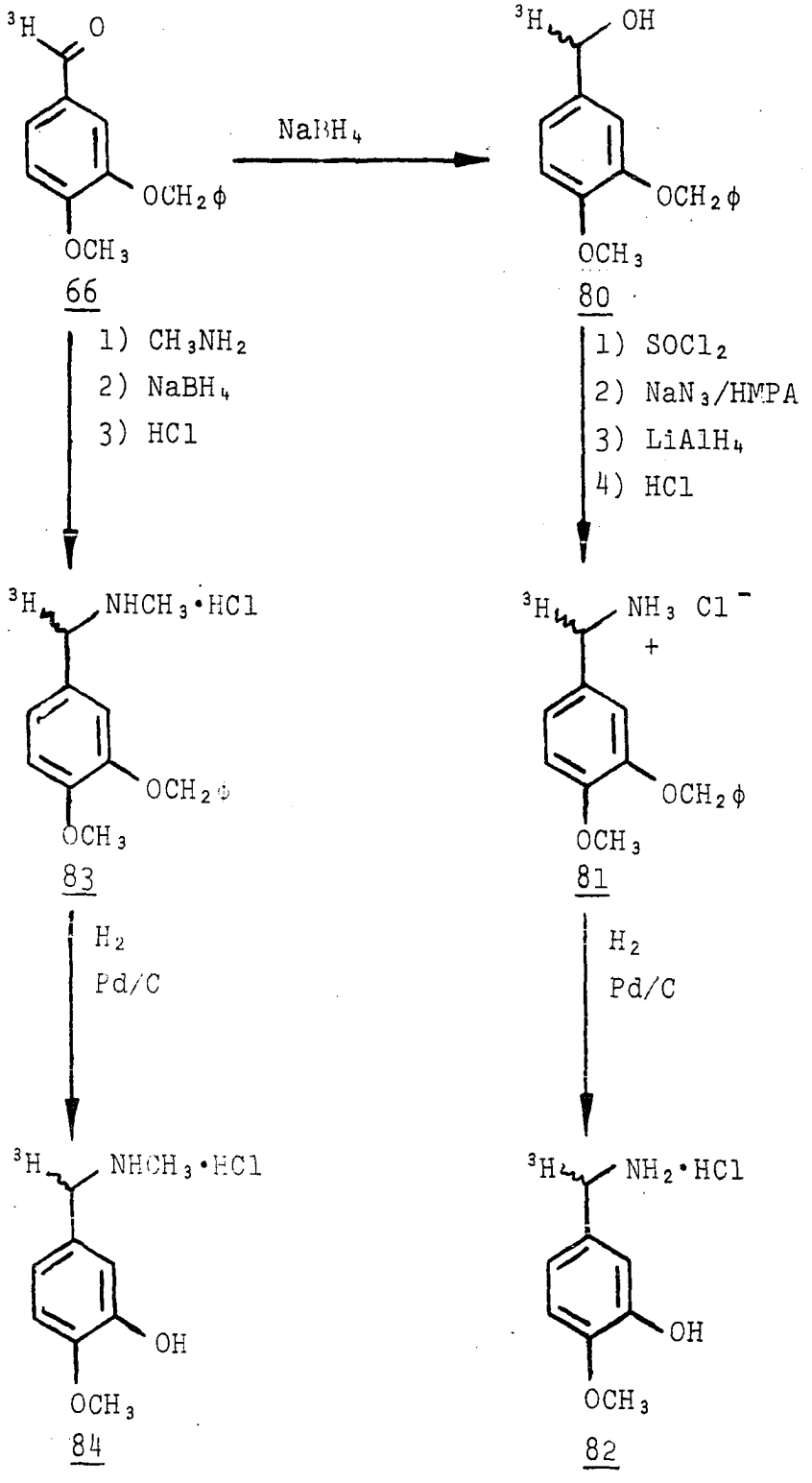


Figure 8. Syntheses of 3-hydroxy-4-methoxy[7-^{3}H]benzylamine hydrochloride and N-methyl-3-hydroxy-4-methoxy-[7-^{3}H]benzylamine hydrochloride.

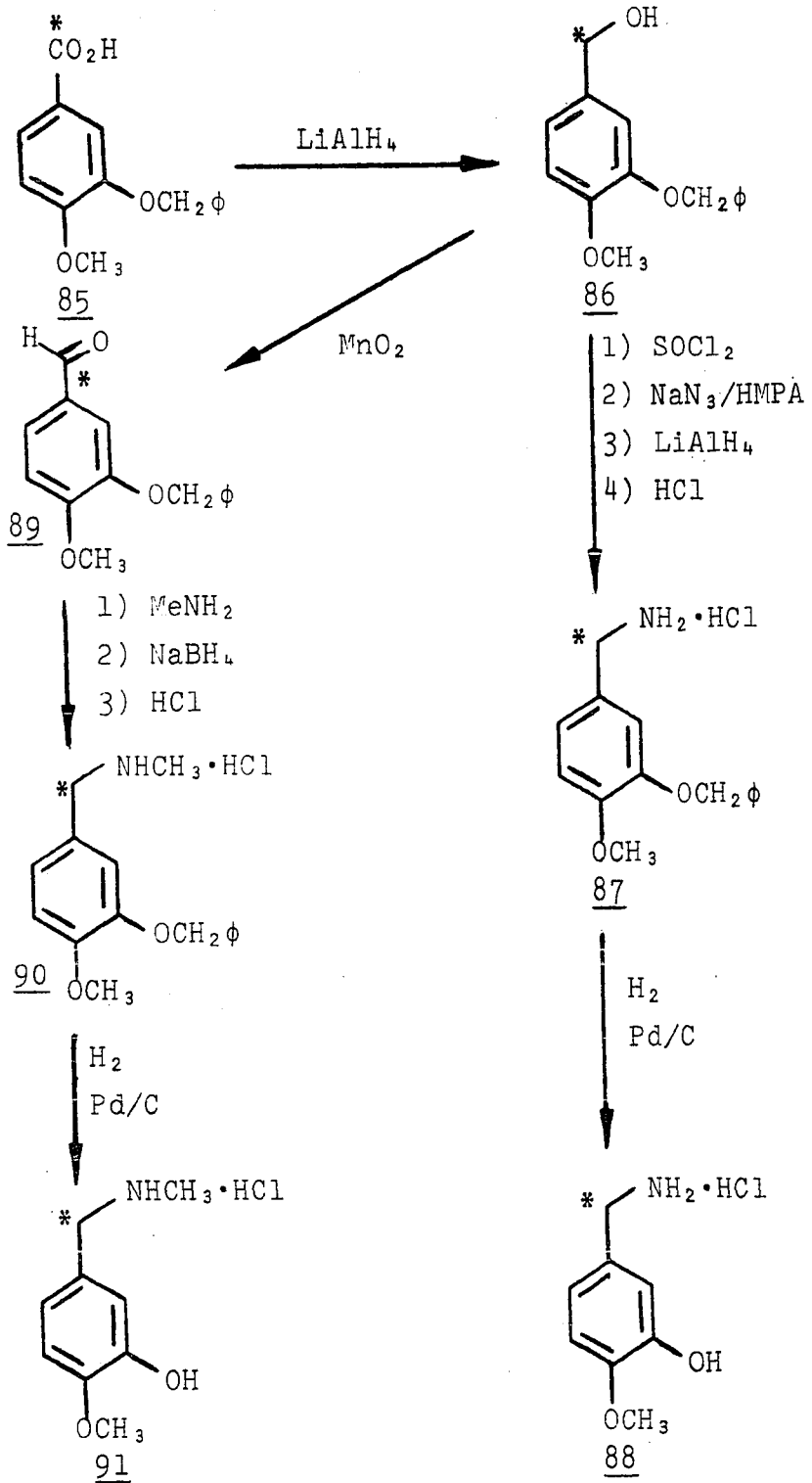
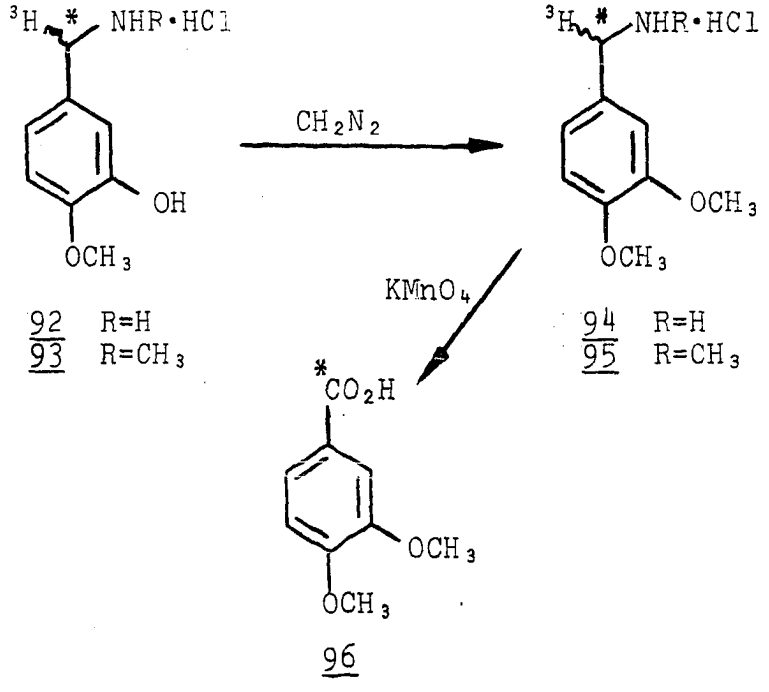


Figure 9. Syntheses of 3-hydroxy-4-methoxy[7-^{14}C]benzylamine hydrochloride and N-methyl-3-hydroxy-4-methoxy-[7-^{14}C]benzylamine hydrochloride.

A small portion of each of the doubly-labeled amine hydrochlorides, (92) and (93), was diluted with the corresponding non-radioactive amine hydrochloride. The diluted amine hydrochlorides (92) and (93) were converted to the corresponding doubly-labeled veratrylamine hydrochlorides (94) and (95) with diazomethane before oxidation with potassium permanganate to veratric acid (96).



92 R=H
93 R=CH_3

94 R=H
95 R=CH_3

96

When N-methyl[7-^3H,^{14}C]veratrylamine hydrochloride (95) (^3H - 2.15 x 10^7 dpm/mmol; ^{14}C - 1.69 x 10^7 dpm/mmol) was oxidized with potassium permanganate, the isolated veratric acid (96) (^3H - 2.91 x 10^3 dpm/mmol; ^{14}C - 1.65 x 10^7 dpm/mmol) contained only 0.01% of the original tritium label indicating

that the tritium label was originally all in the benzylic position. When (94) (^{3}H - 1.24 x 10^{7} dpm/mmol; ^{14}C - 1.28 x 10^{7} dpm/mmol) was oxidized, the isolated veratric acid (96) (^{3}H - 3.38 x 10^{6} dpm/mmol; ^{14}C - 1.24 x 10^{7} dpm/mmol) contained 26.4% of the original tritium label. The amine hydrochloride (94) must have only 73.6% of the tritium label at the benzylic position and the remainder must be distributed throughout the benzene ring and the methoxyl groups. In order to further define the position of the residual tritium label in (96), a Zeisel determination was carried out as described by Miller (11) and Feinstein (7). The isolated methyltriethylammonium iodide was non-radioactive proving that none of the radioactivity was located in the methoxyl groups. The residual tritium activity must therefore be located in the benzene ring of veratric acid (96).

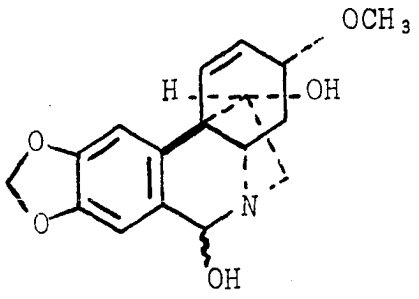
The location of the carbon-14 label at the benzylic position was established by the work of Chan (39).

Biosynthetic Investigations

Feeding experiments with either O-methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride, 3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride, or N-methyl-3-hydroxy-4-methoxy[7-^{3}H, ^{14}C]benzylamine hydrochloride to either Crinum erubescens or Nerine bowdenii

These feeding experiments were carried out using either Crinum erubescens or Nerine bowdenii as the plant hosts.

These plants were chosen because of their availability and their representative alkaloid content. In addition, Crinum erubescens was known to contain 6-hydroxycrinamine (97) and Nerine bowdenii was known to contain 6-hydroxybuphanidrine (98).



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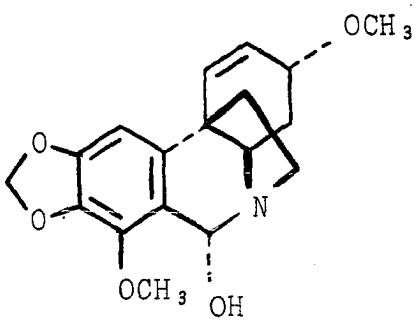


98

All of the doubly-labeled precursors were recrystallized to constant activity as their hydrochloride salts before they were administered to the plant. The precursors were dissolved in 0.5 ml of distilled water and this solution was injected into the bulb. The plants were allowed to grow for three weeks in a laboratory hood under artificial light. After the elapse of three weeks, the bulbs were processed in the standard fashion. The alkaloids were separated and purified by preparative thin layer chromatography and recrystallization. The alkaloids were identified by ir, comparative TLC with authentic samples, and melting point. The alkaloids were recrystallized to constant melting point and constant specific activity.

The specific activities of the radioactive precursors are given in Table 1. The amounts of the isolated alkaloids and the incorporation data determined for each feeding experiment are summarized in Tables 2, 3, 4, and 5. The per cent incorporation for the carbon-14 label and the tritium label are entered separately. The intact incorporation of the precursor is indicated by identical incorporations of each label and by the constancy of each alkaloid's $^{3}H/^{14}C$ ratio when compared to the $^{3}H/^{14}C$ ratio for the precursor.

Interpretation of biosynthetic results

Feeding of O-methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79) to Crinum erubescens and Nerine bowdenii

The data for the incorporation of (79) into Crinum erubescens is summarized in Table 2. Lycorine, crinamine, and 6-hydroxycrinamine were isolated. The constancy of the $^{3}H/^{14}C$ ratios for O-methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79), lycorine hydrochloride, and crinamine established that (79) was incorporated intact into the alkaloids. The observed incorporation data for lycorine hydrochloride and crinamine also proved that the plants were biosynthetically active during the feeding experiment.

In analogy with the observed biosynthetic conversion of haemanthamine (32) to haemanthidine (33) (40,53), 6-hydroxycrinamine (97) would be expected to be biosynthetically derived from crinamine (99). These expectations

Table 1. Precursors for feeding experiments

Compound	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Isotope	$^{3}H/^{14}C$ Ratio
O-Methyl[1'R-^{3}H,1-^{14}C]-norbelladine hydrochloride (79) (*Crinum erubescens*)	7.02	22.1 15.1	155 106	^{3}H ^{14}C	1.46
O-Methyl[1'R-^{3}H,1-^{14}C]-norbelladine hydrochloride (79) (*Nerine bowdenii*)	7.84	22.1 15.1	173 118	^{3}H ^{14}C	1.46
3-Hydroxy-4-methoxy-[7-^{3}H,^{14}C]benzylamine hydrochloride (79) (*Nerine bowdenii*)	6.63	20.1 20.5	133 136	^{3}H ^{14}C	0.98
N-Methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]-benzylamine hydrochloride (93) (*Nerine bowdenii*)	8.35	16.0 12.3	134 103	^{3}H ^{14}C	1.30

Table 2. Summary of incorporation data for alkaloids isolated from a feeding of O-methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79) to Crinum erubescens

Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	^{3}H/^{14}C Ratio
Lycorine hydrochloride	860	2.24×10^{-3} 1.58×10^{-3}	1.93 1.36	1.25 1.28	^{3}H ^{14}C	1.42
Crinamine	135	3.23×10^{-3} 2.24×10^{-3}	0.436 1.303	0.282 0.286	^{3}H ^{14}C	1.44
6-Hydroxycrinamine	34	0.79×10^{-3} 0.88×10^{-3}	0.027 0.030	0.017 0.028	^{3}H ^{14}C	0.90

Table 3. Summary of incorporation data for alkaloids isolated from a feeding of O-methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79) to Nerine bowdenii

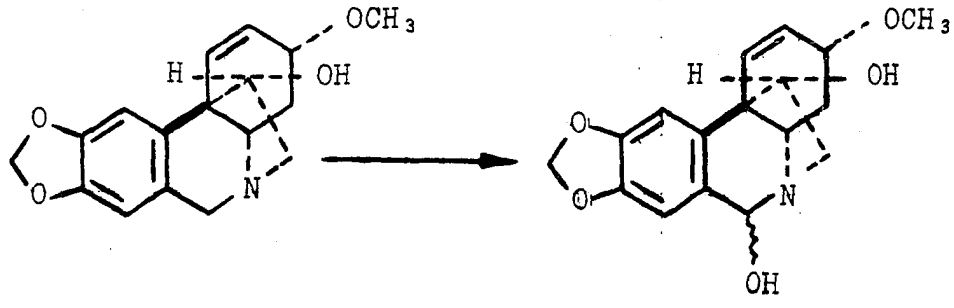
Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	$^{3}H/^{14}C$ Ratio
Lycorine hydrochloride	287	4.21×10^{-3} 2.96×10^{-3}	1.21 0.85	0.705 0.720	^{3}H ^{14}C	1.43
Ambelline	74	0.231×10^{-3} 0.162×10^{-3}	0.017 0.012	0.001 0.001	^{3}H ^{14}C	1.42
Belladine hydrochloride	60	5.83×10^{-2} 4.18×10^{-2}	0.35 0.25	0.202 0.211	^{3}H ^{14}C	1.40
Undulatine	53	1.75×10^{-3} 1.21×10^{-3}	0.09 0.064	0.054 0.054	^{3}H ^{14}C	1.44
6-Hydroxybuphanidrine methiodide	22	0.49×10^{-4} 0.54×10^{-4}	0.011 0.012	0.00064 0.001	^{3}H ^{14}C	0.91

Table 4. Summary of incorporation data for alkaloids isolated from a feeding of 3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (92) to Nerine bowdenii

Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	$^{3}H/^{14}C$ Ratio
Lycorine hydrochloride	146	0	0	0	—	—
Ambelline	73	0	0	0	—	—
Crinamidine	53	0	0	0	—	—
Belladine hydrochloride	7	0	0	0	—	—

Table 5. Summary of incorporation data for alkaloids isolated from a feeding of N-methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (93) to Nerine bowdenii

Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	$^{3}H/^{14}C$ Ratio
Lycorine hydrochloride	143	0	0	0	—	—
Ambelline	53	0	0	0	—	—
Crinamidine	43	0	0	0	—	—
Belladine hydrochloride	35	0	0	0	—	—



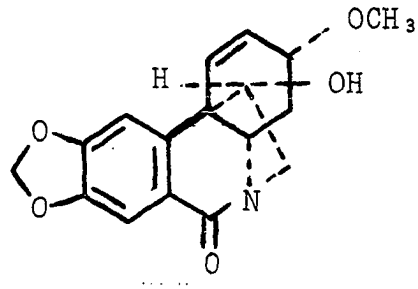
99 97

were consistent with the observed incorporations for the carbon-14 label into (99) and (97). The lower $^{3}H/^{14}C$ ratio observed for 6-hydroxycrinamine (97) relative to the $^{3}H/^{14}C$ ratio observed for the precursor (79), lycorine hydrochloride, and crinamine reflected the loss of a portion of the tritium label during the introduction of the hydroxyl group at C-6 in (97). Specifically, the $^{3}H/^{14}C$ ratio for 6-hydroxycrinamine (97) corresponded to a loss of 36% and retention of 64% of the original tritium label that was at the C-6 position in (97). On the basis of the optical purity determined for O-methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79), loss of the pro-S hydrogen would lead to a $^{3}H/^{14}C$ ratio of 0.96 and loss of the pro-R hydrogen would lead to a $^{3}H/^{14}C$ ratio of 0.45 in the product. The $^{3}H/^{14}C$ ratio observed for 6-hydroxycrinamine (97) corresponds quite well with the $^{3}H/^{14}C$ ratio expected for loss of the pro-S hydrogen atom.

The results for the feeding of O-methyl[1'R-^{3}H,1-^{14}C]-norbelladine hydrochloride (79) to Crinum erubescens establishes that the oxidation at C-6 in 6-hydroxycrinamine involves the loss of the pro-S hydrogen.

This finding is in direct contrast to the findings of Fuganti and Mazza (53,54). Their results predict that the pro-R hydrogen would be lost during the biosynthesis of 6-hydroxycrinamine (97). The resolution of this apparent contradiction must await the publication by Fuganti and Mazza of further experimental details; such as, the specific activities of the precursors, the specific activities of the isolated alkaloids and specific information about the degradations of the isolated alkaloids.

In order to firmly establish the correctness of the results reported in this thesis, it was necessary to carry out the degradation of the isolated 6-hydroxycrinamine (97) in order to confirm the position of the tritium label. After dilution with non-radioactive 6-hydroxycrinamine (97), the diluted (97) (^{3}H - 8.65 x 10^{4} dpm/mmol; ^{14}C - 9.56 x 10^{4} dpm/mmol) was oxidized with freshly prepared activated manganese dioxide (62) to 6-oxocrinamine (100) (^{3}H - 0; ^{14}C - 9.83 x 10^{4} dpm/mmol). The oxidation proceeded with complete loss of the tritium label indicating that the tritium label was all located at the C-6 position in 6-hydroxycrinamine (97).



100

The data for the feeding of O-methyl[1'R-^{3}H,1-^{14}C]-norbelladine hydrochloride (79) to Nerine bowdenii is summarized in Table 3. The incorporation data for this feeding experiment confirms the observations made when (79) was fed to Crinum erubescens. The incorporations and $^{3}H/^{14}C$ ratios for lycorine hydrochloride, ambelline, belladine hydrochloride, and undulatine establish that the plant was biosynthetically active and that (79) was incorporated intact into the alkaloids.

Due to difficulties encountered in trying to crystallize the isolated 6-hydroxybuphanidrine (98), it was converted to its methiodide salt and recrystallized. The rather low incorporation of the carbon-14 label into (98) suggested that it was formed by a relatively minor biosynthetic pathway or by a pathway that was not in operation during the time span of this feeding experiment. The $^{3}H/^{14}C$ ratio of

0.91 for 6-hydroxybuphanidrine (98) was in good agreement with the ratio observed for 6-hydroxycrinamine (97) indicating that the oxidation had occurred with loss of the pro-S hydrogen.

The low specific activity and the small amount of 6-hydroxybuphanidrine methiodide isolated precluded any attempt to carry out a degradation to establish the position of the tritium label.

Feeding of 3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (92) and N-methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (93) to Nerine bowdenii The data for these feeding experiments is summarized in Tables 4 and 5.

In both cases, lycorine hydrochloride, ambelline, crinamidine, and belladine hydrochloride were isolated and a total lack of incorporation of any radioactivity into the alkaloids was observed in any case. This lack of incorporation could not be attributed to a lack of biosynthetic activity on the part of the host plant because the Nerine bowdenii used in the O-methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79) feeding were from the same lot and all of the feeding experiments occurred approximately during the same time span.

The results from these feeding experiments contradict the postulates of Barton and coworkers (18). To explain the

fact that N-[methyl-^{14}C]-3-hydroxy-4-[methoxy-^{14}C]benzylamine (12) was incorporated into haemanthamine (2) and galanthamine (13), Barton and coworkers (18) postulated that (12) was degraded to either isovanillin or to 3-hydroxy-4-methoxybenzylamine before it was incorporated into the alkaloids.

Although caution must be exerted when interpreting negative results because of unknown cellular transport problems, an alternate explanation consistent with both sets of results would be that N-[methyl-^{14}C]-3-hydroxy-4-[methoxy-^{14}C]benzylamine (12) was degraded into "C_1" fragments which could then be incorporated into the alkaloids *via* the "C_1" pool. The conversion of (12) into 3-hydroxy-4-methoxybenzylamine is ruled out by the lack of incorporation of (92). The conversion of (12) to isovanillin is ruled out by the lack of incorporation of either (92) or (93) into the alkaloids.

SUMMARY

O-Methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79) was synthesized in order to study the hydroxylation reaction at the benzylic carbon atom alpha to the nitrogen atom in the Amaryllidaceae alkaloids. 3-Hydroxy-4-methoxy[7-^{3}H,^{14}C]-benzylamine hydrochloride (92) and N-methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (93) were synthesized to examine their possible role as C_6-C_1 precursors for the alkaloids. Both synthetic and chemical degradation procedures for these precursors were discussed. Possible synthetic routes to (R)- or (S)-O-methyl[2-^{3}H]norbelladine were examined.

O-Methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (92) was incorporated intact into 6-hydroxycrinamine (97) and 6-hydroxybuphanidrine (98) with loss of the pro-S hydrogen. The implications of these results were discussed.

3-Hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (92) and N-methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (93) were not incorporated into lycorine, belladine, ambelline, or crinamidine; therefore, (92) and (93) cannot be precursors of the C_6-C_1 unit of the alkaloids.

EXPERIMENTAL

Instrumentation

Infrared spectra were run on a Beckman Model IR-12 or IR-18A spectrophotometer in chloroform solution, or as a potassium bromide pellet, or as a film on a KRS-5 internal reflectance plate (Wilks Scientific). The proton magnetic resonance spectra were run in the indicated solvents with either a Hitachi Perkin-Elmer Model R-20B or a Varian Model A-60 operating at 60 MHz. Melting points were observed on a Köfler hot stage and are corrected. Elemental analyses were carried out by Ilse Beetz, Microanalytical Laboratory, Kronach, West Germany. High resolution mass spectra were obtained through the Department of Chemistry Instrument Services on an AEI MS-902 mass spectrometer. Radioactivities were measured with a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3002) operating at ambient temperature.

All spectral and analytical data for new compounds was obtained on non-radioactive materials.

Radioassay of Compounds

Samples with a high specific activity were dissolved in absolute methanol and three aliquots of 1.0 ml each were counted ten times in 15 ml of toluene: POPOP, PPO [4.9 g of 2,5-diphenyloxazole (PPO) (Packard) and 0.1 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) (Packard) dissolved in

sufficient dry toluene to make 1 l. of solution] and the average value was used. Samples with low specific activities were dissolved directly in 15 ml of Bray's solution (63) [60 g of naphthalene, 4 g of 2,5-diphenyloxazole (PPO) (Packard) and 0.1 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) (Packard) dissolved in 20 ml of ethylene glycol, 100 ml of methanol and sufficient dry, peroxide-free dioxane to make 1 l. of solution] and the average value for ten counts was used.

The two channels of the scintillation counter were adjusted so that the highest counting efficiency without overlap of the tritium counts into the carbon-14 channel was obtained for counting doubly-labeled samples. The tritium counts were corrected for overlapping carbon-14 counts. The overlap of the carbon-14 counts in the tritium channel varied from 18 to 21% in toluene: POPOP, PPO and 40 to 46% in Bray's solution. The counting efficiencies were determined by injecting 50 µl of [^{3}H]toluene or [^{14}C]toluene directly into the counting solution. The tritium counting efficiency varied from 24 to 27% in toluene: POPOP, PPO and 20 to 22% in Bray's solution. The carbon-14 counting efficiency varied from 29 to 32% in Bray's solution and 44 to 49% in toluene: POPOP, PPO.

All compounds were recrystallized to constant activity. The alkaloids were identified by comparative TLC in at least two solvent systems, by melting point, by mixed melting point, and by comparison of their ir spectra with known reference spectra. The per cent incorporation was calculated as 100 x [total activity of the isolated pure alkaloid] ÷ [total activity fed].

Thin Layer Chromatography

The alkaloids were separated by preparative scale thin layer chromatography (TLC) on 20 cm x 20 cm glass plates with a 0.5 mm layer of silica gel (Merck, PF_{254}) containing an ultraviolet indicator. The TLC plates were activated by placing them in an oven (100°) for at least 12 hours. Four general solvent systems were used for carrying out the separations; solvent A [95 parts chloroform/3 parts methanol/ 2 parts diethylamine (v/v/v)], solvent B [90 parts chloroform/5 parts methanol/ 5 parts diethylamine (v/v/v)], solvent C [70 parts ethyl acetate/30 parts methanol (v/v)], and solvent D [85 parts diethyl ether/10 parts methanol/ 5 parts diethylamine (v/v/v)]. The separated compounds were detected by illumination of the TLC plate with a 254 nm lamp and scraped from the plate. The silica gel was soaked in methanol overnight. To recover the compounds, the methanol slurry was filtered, the methanol was removed <u>in vacuo</u>, and

the residue was dissolved in chloroform. The chloroform solution was filtered and evaporated *in vacuo*. The recovered compound was then weighed and recrystallized.

Attempted Syntheses of (R)- or (S)-O-Methyl[2-^{3}H]norbelladine

Z-1-Methyl-2-phenylcyclopropanecarboxylic acid (47)

This compound (47) was prepared by the method of Depuy and coworkers (55): mp 81-82° (lit. (55) 80.5-81°).

Z-1-Methyl-2(p-nitrophenyl)cyclopropanecarboxylic acid (48)

During a one hour period, (47) (15 g) was carefully added to 100 ml of concentrated nitric acid at 25°. The mixture was then placed in a freezer and allowed to stand for 36 hours. The crystals which had formed were quickly filtered out of the mixture and washed with water. The crystals were recrystallized from ethanol-water to give 8.4 g (45%) of yellow needles: mp 119-121°. The material was sublimed at 95° (0.005 mm). The sublimed material melted at 152°: ir ($CHCl_3$) 1695 (s) (C=O), 1605 (m), 1527 (s), 1350 (s), 900 (m), and 860 cm^{-1}; nmr ($CDCl_3$) δ 1.0 (s, 3, CH_3), 1.3 (q, 1, CH), 1.9 (q, 1, CH), 2.97 (q, 1, CH), 7.8 (q, 4, aromatic), and 12.2 (s, 1, CO_2H).

Anal. Calcd. for $C_{11}H_{11}NO_4$: C, 59.72; H, 5.01; N, 6.32. Found: C, 59.67; H, 4.96; N, 6.22.

Ethyl Z-1-methyl-2-(p-nitrophenyl)cyclopropanecarboxylate (49)

The acid (48) (5.01 g) was dissolved in 150 ml of absolute ethanol saturated with anhydrous hydrogen chloride. The resultant solution was refluxed for 24 hours and then the solvent was evaporated in vacuo. The oily residue was crystallized from benzene-hexane as yellow crystals; 5.3 g (95%): mp 50-55°. Sublimation at 45° (0.005 mm) yielded large yellow needles: mp 55-57°; ir ($CHCl_3$) 1715 (s) (C=O), 1600 (m), 1530 (s), 1350 (s), 1180 (m), and 860 (m) cm^{-1}; nmr ($CDCl_3$) δ 1.0 (s, 3, CH_3), 1.35 (t, 3, $-CH_2CH_3$), 1.35 (q, 1, CH), 1.8 (q, 1, CH), 2.9 (q, 1, PhCH), 4.2 (q, 2, OCH_2), and 7.77 (q, 4, aromatic).

Anal. Calcd. for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.05; N, 5.61. Found: C, 62.67; H, 5.98; N, 5.48.

Ethyl Z-1-methyl-2-(p-aminophenyl)cyclopropanecarboxylate hydrochloride (50)

A mixture of (49) (5.1 g) and granulated tin (7.09 g) in 75 ml of absolute ethanol was heated to near reflux. Concentrated hydrochloric acid (15 ml) was added slowly to the mixture, which was stirred at 70° for 2 hours. After refluxing the mixture for an additional hour, approximately 90% of the ethanol was distilled from the reaction mixture. Water (200 ml) was added to the residue and the aqueous solution was extracted with chloroform. The chloroform layer was washed twice with 10% sodium hydroxide. The chloroform

solution was dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The residual oil was converted to its hydrochloride salt and recrystallized from ethanol-ether, yield 4.7 g (80%): mp 119-121°; ir ($CHCl_3$) 3000 (s), 2600 (m), 1718 (s) (C=O), 1520 (s), 1185 (s), and 912 (s) cm^{-1}; nmr ($CDCl_3$) δ 0.93 (s, 3, CH_3), 1.1 (q, 1, CH), 1.3 (t, 3, CH_2CH_3), 1.7 (q, 1, CH), 2.77 (q, 1, PhCH), 4.15 (q, 2, OCH_2), 7.33 (q, 5, aromatic), and 10.3 (broad, 3, NH_3Cl).

Anal. Calcd. for $C_{13}H_{18}NO_2Cl$: C, 61.04; H, 7.07; N, 5.47. Found: C, 60.90; H, 6.91; N, 5.33.

Z-1-Methyl-2-(p-hydroxyphenyl)cyclopropanecarboxylic acid (51)

A solution of (50) (3.27 g) in 75 ml of 6 N sulfuric acid was cooled to 0° and sodium nitrite (1.60 g) in 15 ml of water was added slowly to the reaction mixture. After stirring for an hour at 0-5°, the reaction mixture was added to 200 ml of 10% sulfuric acid at 75°. After the evolution of nitrogen had ceased, the aqueous mixture was cooled and extracted with diethyl ether. The ether layer was dried with sodium sulfate and concentrated *in vacuo*. The ether concentrate was passed through a six-inch silica gel column. The ethereal eluate was evaporated to dryness *in vacuo*. The residue crystallized from benzene, yield 1.05 g (37%): mp 148-149°; ir (KRS-5) 3400 (m), 1690 (s) (C=O), 1620 (m), 1513 (s), 1460-1420 (m),

1250 (s), 1200 (s), 965 (m), 910 (m), and 845 (m) cm^{-1}; nmr (d_6-acetone) δ 0.95 (s, 3, CH_3), 1.18 (q, 1, CH), 1.63 (q, 1, CH), 2.7 (q, 1, PhCH), 6.9 (q, 4, aromatic), and 8.85 (broad, 2, CO_2H, OH).

Anal. Calcd. for $C_{11}H_{12}O_3$: C, 68.72; H, 6.29. Found: C, 68.71; H, 6.49.

Z-1-Methyl-2-(p-methoxyphenyl)cyclopropanecarboxylic acid (52)

Dimethyl sulfate (1.13 g) was added slowly to a solution of potassium hydroxide (0.50 g) and (51) (0.864 g) in 25 ml of methanol. After refluxing the reaction for one hour, water (100 ml) was added to the reaction mixture and 50 ml of solvent was distilled from the reaction mixture. The reaction mixture was cooled, acidified to pH 5, and extracted with diethyl ether. The ether solution was dried with sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was recrystallized from benzene, yield 0.743 g (80%): mp 115-116.5°; ir ($CHCl_3$) 3020 (s), 2580 (m), 1685 (s) (C=O), 1610 (m), 1515 (s), 1465 (m), 1425 (m), 1305 (m), 1250 (s), 1050 (m), 1030 (m), 960 (m), 900 (m), and 840 (s) cm^{-1}; nmr ($CDCl_3$) δ 0.95 (s, 3, CH_3), 1.15 (q, 1, CH), 1.75 (q, 1, CH), 2.85 (q, 1, PhCH), 3.73 (s, 3, OCH_3), 6.95 (q, 4, aromatic), and 11.5 (s, 1, CO_2H).

M.S. Calcd. for $C_{12}H_{14}O_3$: 206.0942. Found: 206.0940.

E-Acetyl-1-methyl-2(p-methoxyphenyl)cyclopropane (53)

Thirty-four milliliters of a 0.6 M solution of methyllithium in diethyl ether was added over a period of 30 minutes to a magnetically stirred solution of 2.01 g of (52) in 50 ml of diethyl ether. The reaction mixture was stirred at room temperature for one hour and 50 ml of a saturated solution of ammonium chloride was added carefully. After the phases had separated, the aqueous layer was extracted with diethyl ether. The ether fractions were combined, dried with magnesium sulfate, filtered, and evaporated to dryness in vacuo. The residue was distilled through a short path distillation column, yield 1.65 g (85%): bp 95-96° (0.075 mm); ir (thin film) 1690 (s) (C=O), 1610 (m), 1515 (s), 1460 (m), 1250 (s), 1180 (m), 1045 (s), and 835 (s) cm^{-1}; nmr ($CDCl_3$) δ 1.05 (s, 3, CH_3), 1.18 (q, 1, CH), 1.7 (q, 1, CH), 2.2 (s, 3, $COCH_3$), 2.7 (q, 1, PhCH), 3.76 (s, 3, OCH_3), and 7.0 (q, 5, aromatic).

M.S. Calcd. for $C_{13}H_{16}O_2$: 204.1149. Found: 204.1150.

Baeyer-Villiger rearrangement of (53)

Trifluoroperacetic acid was prepared by dropwise addition of 2.75 ml of 90% hydrogen peroxide to 15.3 g of trifluoroacetic anhydride in 75 ml of methylene chloride at 0-10°. The solution was added to a stirred slurry of 125 g of anhydrous, granular Na_2HPO_4 and 4.96 g of (53) in 150 ml

of methylene chloride at 10°. The reaction mixture was allowed to warm to room temperature over a period of 5 hours. The solids were filtered and washed twice with 50 ml of methylene chloride. The methylene chloride fractions were combined, washed with 100 ml of a saturated sodium bicarbonate solution, then with 100 ml of saturated sodium chloride solution, and dried with magnesium sulfate. The methylene chloride solution was evaporated *in vacuo* to give 5.3 g of a multi-component mixture. Chromatography of 3.3 g of the mixture on a silica gel column gave two major fractions. Fraction A (1.5 g) was eluted with hexane and fraction B (1.0 g) was eluted with chloroform:methanol (50:50). Fraction B was soluble in 10% sodium hydroxide and gave a positive test for phenols when tested with 1% ferric chloride solution (64). Fraction A was chromatographed on silica gel plates (TLC) [benzene:acetone (90:10)]. The major fraction (0.827 g) (R_f=0.7) was an oil. The major component (by nmr) was anisaldehyde (53b). The 2,4-DNP derivative was prepared: mp 250-255°d. (Lit. (65) 254°d.)

Identical results were obtained when permaleic acid was substituted for trifluoroperacetic acid. When either *m*-chloroperbenzoic acid, perbenzoic acid, or 40% peracetic acid were substituted for trifluoroperacetic acid, the starting material (53) was recovered quantitatively even after stirring at room temperature for periods of 6 to 24 hours.

p-Benzyloxymandelic acid (58)

The synthesis of (58) was patterned after the approach of Compere (58) to α-hydroxy arylacetic acids. To a mixture of 0.5 m of lithium chloride, 1.0 m of potassium hydroxide, and 200 g of ice was added 200 ml of dioxane, 0.25 m of p-benzyloxybenzaldehyde (57) and 0.25 m of bromoform. The mixture was stirred for 24 hours at 4° and then the pH was adjusted to pH 12-13. The mixture was stirred at room temperature for 24 hours. After dilution of the reaction mixture with 600 ml of water, the precipitate was filtered and the filtrate was extracted twice with chloroform. The filtrate and precipitate were recombined before the pH of the mixture was adjusted to pH 1. The aqueous mixture was extracted with ethyl acetate. The ethyl acetate extract was dried with magnesium sulfate and evaporated to dryness in vacuo. The residue was dissolved in a minimum volume of hot ethyl acetate and an equal volume of benzene. Upon cooling, fine white needles were obtained, yield 39.5 g (62%): mp 160-161°; ir (KBr) 3460 (s), 3100-2500 (s), 1745 (s) (C=O), 1310-1250 (s), 1190 (s), 1090 (s), 1070 (s), 1020 (m), 940 (m), 865 (m), and 830 (m) cm^{-1}; nmr (d_4-methanol) δ 4.7 (s, H_2O, CO_2H, OH), 4.95 (s, 2, $PhCH_2$), 5.0 (s, 1, CHOH), 7.05 (q, 4, aromatic), and 7.23 (s, 5, aromatic).

M.S. Calcd. for $C_{15}H_{14}O_4$: 258.0892. Found: 258.0888.

Resolution of (58)

A solution of (58) (26.43 g) and cinchonine (30.11 g) in 800 ml of acetone:chloroform (2:1) was allowed to slowly evaporate until a volume of approximately 150 ml was attained and crystallization had begun. The solution was then cooled for 24 hours. The crystals were filtered from the solution and recrystallized to a constant rotation, $[\alpha]_D^{25} = 83°$ (methanol) and melting point, 168-169°, yield 8.09 g. The pure salt (7.43 g) was dissolved in a two phase system composed of 200 ml of 10% hydrochloric acid and 200 ml of ethyl acetate. The layers were separated and the aqueous layer was extracted five times with ethyl acetate. The ethyl acetate fractions were combined, dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was crystallized from ethyl acetate-benzene, yield 3.82 g: mp 161-162°; $[\alpha]_D^{25} = -101.5°$ (methanol).

(-)-Methyl p-benzyloxymandelate (59)

An ethereal solution containing a 10 fold excess of diazomethane was added to a solution of (-)-(58) (3.0 g) in 50 ml of methanol. The solution was allowed to stand at room temperature for 24 hours. The solvent was evaporated with a stream of dry nitrogen and the residue was crystallized from benzene-hexane, yield 3.14 g (99%): mp 72-74°; $[\alpha]_D^{25} = -135.8°$ (benzene); ir ($CHCl_3$) 3500 (w), 1730 (s)

(C=O), 1610 (m), 1510 (m), 1240 (s), 1210 (s), and 1080 (m) cm^{-1}; nmr ($CDCl_3$) δ 3.7 (s, 3, OCH_3), 5.0 (s, 2, $PhCH_2$), 5.08 (s, 1, CHOH), 7.08 (q, 4, aromatic), and 7.32 (s, 5, aromatic).

M.S. Calcd. for $C_{16}H_{16}O_4$: 272.1048. Found: 272.1044.

(-)-2-Hydroxy-2-(p-benzyloxyphenyl)ethanol (60)

A solution of 5.0 g of (59) in 25 ml of dry tetrahydrofuran was added slowly to a slurry of 2.0 g of lithium aluminum hydride in 25 ml of dry tetrahydrofuran. The reaction mixture was refluxed for 12 hours and the excess lithium aluminum hydride was destroyed by Fieser's method (66) with water:base:water. The alumina was filtered from the solution and the solvent was evaporated in vacuo. The residue was recrystallized from benzene, yield 4.3 g (98%): mp 131-132°; $[\alpha]_D^{25}$ = -27.2° (methanol); ir (KBr) 3400 (s), 1620 (m), 1525 (m), 1475 (m), 1385 (m), 1260 (s), 1180 (m), 1090 (s), 1023 (s), and 825 (s) cm^{-1}; nmr (d_6-acetone) δ 3.55 (d, 2, CH_2OH), 3.9 (broad, 2, OH), 4.63 (t, 1, CHOH), 5.5 (s, 2, $PhCH_2$), 7.10 (q, 4, aromatic), and 7.35 (s, 5, aromatic).

M.S. Calcd. for $C_{15}H_{16}O_3$: 244.1099. Found: 244.1102.

2-Hydroxy-2-(p-benzyloxyphenyl)ethyl trityl ether (61)

A solution of 1.56 g of (60) and 7.27 g of trityl chloride in 25 ml of dry pyridine was stirred for 48 hours at room

temperature. The mixture was heated on a steam bath for two hours and the pyridine was evaporated *in vacuo*. The residue was dissolved in ethyl acetate and then washed with 3% hydrochloric acid. The ethyl acetate solution was dried with magnesium sulfate, filtered, and evaporated to dryness *in vacuo*. The residue was chromatographed on a Florisil column. Triphenylcarbinol was eluted first with hexane and (61) was eluted with hexane:benzene (75:25). The solvent was evaporated to dryness *in vacuo*. The residue was crystallized from hexane, yield 1.45 g (47%): mp 108-110°; $[\alpha]_D^{25}$ = 0°; ir ($CHCl_3$) 3600 (m), 3010 (m), 1610 (s), 1520 (s), 1495 (m), 1450 (s), 1250 (s), 1170 (s), 1060 (s), 1030 (m), and 830 (m) cm^{-1}; nmr ($CDCl_3$) δ 2.6 (broad, 1, OH), 3.28 (d, 2, CH_2O), 4.7 (t, 1, CHOH), 4.95 (s, 2, $PhCH_2$), and 6.7-7.5 (m, 24, aromatic).

Anal. Calcd. for $C_{34}H_{30}O_3$: C, 83.95; H, 6.17. Found: C, 84.05; H, 6.16.

The starting material was eluted from the Florisil column with methanol. Evaporation of the methanol *in vacuo* and crystallization of (60) from ethyl acetate yielded 0.36 g: mp 130-131°; $[\alpha]_D^{25}$ = -6.08° (methanol).

2-(p-Benzyloxyphenyl)ethyl trityl ether (62)

A solution of 0.60 g of thionyl chloride in 25 ml of dry ether was added to a solution of 1.12 g of (61) and 0.4 g of dry pyridine in 25 ml of dry ether at 0° over a

period of 30 minutes. The solution was stirred at 0° for 5 hours and then at room temperature for one hour. The mixture was poured into 50 ml of ice water and extracted with ethyl acetate. The organic layer was washed with 10% sulfuric acid, saturated sodium bicarbonate solution, and saturated sodium chloride solution. The ethyl acetate solution was dried with magnesium sulfate, filtered, and evaporated to dryness *in vacuo* to yield 1.00 g of crude chloride. A mixture of the crude chloride (0.51 g) and lithium aluminum hydride (0.155 g) in 25 ml of dry diethyl ether was refluxed for 48 hours. The excess hydride was destroyed by addition of water:base:water (66). The precipitated alumina was filtered from the solution. The diethyl ether solution was dried with magnesium sulfate, filtered, and evaporated to dryness *in vacuo*. The residue crystallized from hexane, yield 0.47 g (85%): mp 93-95°; ir ($CHCl_3$) 3010 (m), 1615 (m), 1515 (s), 1495 (m), 1450 (s), 1250 (s), 1070 (m), 1030 (m), and 1010 (m) cm^{-1}; nmr ($CDCl_3$) δ 2.7 (t, 2, $CH_2\underline{CH_2}O$), 3.15 (t, 2, $\underline{CH_2}CH_2O$), 4.87 (s, 2, $Ph\underline{CH_2}$), and 6.7-7.4 (m, 24, aromatic).

M.S. Calcd. for $C_{34}H_{30}O_2$: 470.2246. Found: 470.2240.

2-(p-Benzyloxyphenyl)ethanol (63)

A solution of 0.100 g of (62) in 1 ml of benzene was placed on a column of Woelm silica gel (act. 1, 2.5 g) and allowed to stand for 36 hours. Triphenylcarbinol was eluted

from the column with benzene. 2-(p-Benzyloxyphenyl)ethanol (63) was eluted with ethyl acetate. The ethyl acetate solution was evaporated in vacuo and the residue was crystallized from hexane, yield 0.047 g (97%): mp 83-84°; ir ($CHCl_3$) 3620 (w), 1515 (s), 1250 (m), 1180 (m), 1045 (m), and 1030 (m) cm^{-1}; nmr ($CDCl_3$) δ 1.5 (broad, 1, OH), 2.7 (t, 2, CH_2OH), 3.72 (t, 2, CH_2CH_2OH), 4.93 (s, 2, $PhCH_2$), 6.86 (q, 4, aromatic), and 7.23 (s, 5, aromatic).

M.S. Calcd. for $C_{15}H_{16}O_2$: 228.1150. Found: 228.1162.

Attempted racemization of (60)

Run A: The diol (60) (100 mg) ($[\alpha]_D^{25}$ = -27.1° (methanol)) was dissolved in 10 ml of dry pyridine and stirred at room temperature for 48 hours. The pyridine was evaporated in vacuo. The residue was dissolved in ethyl acetate and then washed with 3% hydrochloric acid. The ethyl acetate solution was dried with magnesium sulfate, filtered, and concentrated in vacuo. Upon cooling, the diol (60) crystallized, yield 87 mg: mp 130-131°; $[\alpha]_D^{25}$ = -26.2° (methanol).

Run B: The procedure outlined for Run A was repeated and in addition, the solution of (60) was heated on a steam bath for three hours. After workup of the solution as described for Run A, the diol (60) (93 mg) was recovered: mp 130-131°; $[\alpha]_D^{25}$ = -27.8° (methanol).

Run C: Run B was repeated with the addition of 48 mg of pyridine hydrochloride to the solution of (60) in pyridine. The reaction was worked up as previously described and the diol (60) (78 mg) was recovered: mp 131-132°; $[\alpha]_D^{25}$ = -26.8° (methanol).

Run D: The diol (60) (100 mg) was chromatographed on a Woelm silica gel (act. 1) column and eluted with chloroform: methanol (50:50). The solvent was evaporated to dryness *in vacuo*. The residue was crystallized from ethyl acetate, yield 93 mg: mp 130-131°; $[\alpha]_D^{25}$ = -27.2° (methanol).

Synthesis of O-Methyl[1'R-^{3}H,1-^{14}C]norbelladine Hydrochloride

O-Benzylisovanillin (64)

O-Benzylisovanillin (64) was prepared from isovanillin by the method of Robinson and Sugasawa (67): mp 57-60° (lit. (67) 60-61°).

3-Benzyloxy-4-methoxyphenyl morpholino acetonitrile (65)

The morpholino acetonitrile was prepared by the method of Bennett and coworkers (68). A mixture of O-benzylisovanillin (64) (2.42 g) and morpholine perchlorate (1.87 g) was heated in 10 ml of morpholine at 60° for one hour. Sodium cyanide (0.539 g) was dissolved in 1 ml of water and added to the mixture which was then heated at 90° for one

hour. Addition of cold water (50 ml) to the cooled reaction mixture precipitated the crude product, which was filtered from the solution and dissolved in diethyl ether. The ethereal solution was then dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The product was crystallized from diethyl ether-hexane, yield 2.18 g (65%): mp 100-101°; ir ($CHCl_3$) 2240 (m), 1500 (m), 1450 (m), 1250 (s), 1130 (s), 1105 (s), 1010 (s), and 905 (s) cm^{-1}; nmr ($CDCl_3$) δ 2.45 (t, 4, $C\underline{H}_2N$), 3.65 (t, 4, $C\underline{H}_2O$), 3.9 (s, 3, $OC\underline{H}_3$), 4.68 (s, 1, $C\underline{H}CN$), 5.2 (s, 2, $PhC\underline{H}_2$), and 6.8-7.5 (m, 8, aromatic).

M.S. Calcd. for $C_{20}H_{22}N_2O_3$: 338.1360. Found: 338.1367.

O-Benzyl[7-^{3}H]isovanillin (66)

The labeling experiment was performed in a manner analogous to that described by Bennett and coworkers (68). Sodium hydride (0.144 g of a 50% oil dispersion) was added with stirring to a solution of (65) (3.38 g) in 20 ml of dry dimethylformamide under a dry, oxygen free, nitrogen atmosphere. After stirring for one hour, tritiated water (0.20 ml, spec. act. = 1C/ml) was added. The reaction mixture was stirred for 15 minutes and then cooled to 0°. The reaction mixture was treated with one equivalent of thionyl chloride and then quickly poured into 100 ml of water. The product (65) was extracted with chloroform.

The chloroform solution was dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The residue was recrystallized from ether, yield 3.30 g (98%): mp 100-101°. The labeled morpholino acetonitrile (65) was hydrolyzed by refluxing 300 mg of (65) in 25 ml of 1 N hydrochloric acid and 8 ml of tetrahydrofuran for 30 minutes. After cooling, the reaction mixture was extracted with chloroform. The chloroform solution was washed with 10% sodium hydroxide, dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The residue was crystallized from diethyl ether-hexane, yield 129-170 mg (60-79%): mp 57-60° (lit. (67) 60-61°).

Degradation of (66)

Approximately one milligram of (66) was diluted with one gram of non-radioactive O-benzylisovanillin (64) and recrystallized to constant activity (^3H - 2.39 x 10^5 dpm/mg). A solution of potassium permanganate (360 mg) in 20 ml of water was added to a stirring solution of the diluted aldehyde (66) (550 mg) in 20 ml of dioxane. After stirring for one hour, sulfur dioxiode was bubbled through the solution to remove manganese dioxide. The solution was extracted with chloroform. The chloroform solution was extracted with 10% sodium hydroxide. The sodium hydroxide solution was cooled, acidified with concentrated sulfuric acid, and extracted with chloroform. The chloroform solution

was dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The residue was crystallized from ethanol to yield 250 mg (43%) of 3-benzyloxy-4-methoxybenzoic acid: mp 177-179° (lit. (69) 177°); (^{3}H - 3.64 x 10^{1} dpm/mg).

3-Benzyloxy-4-methoxy[7S-^{3}H]benzyl alcohol (67)

The S-alcohol was prepared by the method of Battersby (70). The aldehyde (66) (251 mg) was dissolved in 20 ml of freshly distilled absolute ethanol and 5 ml of this solution was added to a mixture of distilled water (350 ml), glycine buffer (50 ml, 0.05 N, pH 8.8), nicotinamide-adenine dinucleotide (NAD) (150 mg), absolute ethanol (20 ml), 3 N sodium hydroxide (a few drops to adjust the pH to between pH 8 and pH 9), and equine liver alcohol dehydrogenase (LADH) (25 mg) at a temperature of 37.5°. The remaining aldehyde (66) was added in 5 ml aliquots at 30 minutes, 60 minutes, and 105 minutes. After 6 hours, the reaction mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo* without heating. The residue (234 mg) was chromatographed on silica gel TLC plates, which were developed with chloroform. The alcohol (67) was recovered from the most polar band on the plate and crystallized from benzene-hexane, yield 214 mg (85%):

mp 72-73° (lit. (39) 76°); ir ($CHCl_3$) 3600 (w), 1520 (s), 1465 (m), 1450 (m), 1435 (m), 1390 (m), 1270 (s), 1145 (s), and 1030 (s) cm^{-1}.

3-Benzyloxy-4-methoxy[7R-^{3}H]benzylamine hydrochloride (68)

A solution of the (S)-alcohol (67) (300 mg) and colorless thionyl chloride (0.45 ml) in 10 ml of dry, freshly distilled dioxane was allowed to stir at room temperature for 30 minutes. The solvent was evaporated in vacuo with very gentle heating. The residue was dissolved in 10 ml of dry diethyl ether and the solution was evaporated to dryness in vacuo. The residue was dissolved in 10 ml of dry hexamethylphosphoric triamide and added to a slurry of 1.65 g of sodium azide in 25 ml of dry hexamethylphosphoric triamide at 60°. The mixture was stirred at 60° for 6 hours. After addition of water (250 ml), the mixture was extracted with diethyl ether. The ether solution was washed with water, saturated brine solution, dried with sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was dissolved in 10 ml of dry diethyl ether and added to a refluxing slurry of lithium aluminum hydride (150 mg) in 25 ml of dry diethyl ether. After refluxing for 3 hours, a 25 ml portion of a saturated sodium potassium tartrate solution was added to the reaction mixture. The layers were separated and the aqueous layer was extracted with diethyl

ether. The diethyl ether solution was washed with saturated sodium chloride solution, dried with sodium sulfate, filtered, and evaporated to an oil *in vacuo*. The oil was dissolved in 5 ml of absolute ethanol and anhydrous ethereal hydrogen chloride was added. The amine hydrochloride (68) crystallized as white plates, yield 227 mg (69%): mp 244.5-246°; ir (KBr) 3400 (w), 3000 (s) 1530 (m), 1270 (s), and 1025 (m) cm^{-1}; nmr (d_4-methanol) δ 3.83 (s, 3, OCH_3), 4.0 (s, 2, CH_2N), 4.77 (s, H_2O, NH_3), 5.10 (s, 2, $PhCH_2$), and 7.0-7.6 (m, 8, aromatic).

M.S. Calcd. for $C_{15}H_{17}NO_2$: 243.1254. Found: 243.1259.

N-3-Benzyloxy-4-methoxy[7R-^{3}H]benzyl 4-benzyloxyphenylacetamide (69)

A mixture of 10 ml of 10% sodium hydroxide and 20 ml of benzene was heated at 60° and (68) (200 mg) was added with stirring. After (68) was completely dissolved, a solution of p-benzyloxyphenacetyl chloride (363 mg) in 20 ml of benzene was added to the stirring mixture. After one hour, the two phases were separated. The benzene solution was washed with 10% hydrochloric acid, dried with sodium sulfate, and evaporated to dryness *in vacuo*. The residue was crystallized from benzene, yield 305 mg (92%): mp 136-138°; ir ($CHCl_3$) 1665 (s) (C=O), 1525 (s), 1260 (s), 1143 (m), and 1030 (m) cm^{-1}; nmr ($CDCl_3$) δ 2.0 (broad, 1, NH), 2.7

(s, 2, CH_2CO), 3.63 (s, 2, CH_2N), 3.8 (s, 3, OCH_3), 4.95 (s, 2, $PhCH_2$), 5.08 (s, 2, $PhCH_2$), and 6.7-7.5 (m, 17, aromatic).

M.S. Calcd. for $C_{30}H_{29}NO_4$: 467.2096. Found: 467.2128.

O-Dibenzyl-O-methyl[1'R-^{3}H]norbelladine hydrochloride (70)

A solution of the amide (69) (50 mg) was dissolved in 2 ml of dry tetrahydrofuran and added to a refluxing slurry of lithium aluminum hydride (150 mg) in 25 ml of dry diethyl ether over a period of one-half hour. The mixture was refluxed for an additional 5 hours before the excess hydride was destroyed by addition of 25 ml of a saturated solution of sodium potassium tartrate. The layers were separated and the aqueous layer was extracted with diethyl ether. The combined ether extracts were washed with saturated sodium chloride solution and dried with sodium sulfate. After filtering, the solution was evaporated to an oil *in vacuo*. The product from four reductions was collected and purified by TLC on silica gel plates developed in solvent C. The band (R_f=0.6) was collected and the amine was recovered. The amine was converted to its hydrochloride salt and crystallized from ethanol-ether, yield 60 mg (33%): mp 124-126° (lit. (18) 123-124°); ir ($CHCl_3$) 3400 (w), 2980 (m), 1510 (s), 1455 (m), 1250 (s), and 1020 (m) cm^{-1}.

O-Methyl[1'R-^{3}H]norbelladine hydrochloride (71)

The amine hydrochloride (70) was debenzylated by refluxing a solution of (70) (60 mg) in 7 ml of concentrated hydrochloric acid:methanol (6:1) under a nitrogen atmosphere for 3 hours. The solution was cooled and adjusted to pH 8 with concentrated ammonium hydroxide. The aqueous solution was extracted with chloroform. The chloroform solution was dried with sodium sulfate, filtered, and concentrated *in vacuo*. The amine was purified by TLC on silica gel plates developed in solvent B. The band (R_f=0.3) was scraped off and the amine was recovered. The amine was converted to its hydrochloride salt and crystallized from ethanol-ether, yield 22.6 mg (60%): mp 202-205° (lit. (18) 205-207°). The product was shown to be identical to authentic O-methylnorbelladine by comparative TLC in solvents A, B, and C.

Proof of Configuration and Optical Purity of (68)

N,N,N-Trimethyl-(3-benzyloxy-4-methoxy[7R-^{3}H]benzyl)ammonium iodide (72)

A 1 mg portion of (68) was diluted with 400 mg of non-radioactive amine hydrochloride. The diluted (68) was dissolved in 15 ml of methanol containing 480 mg of sodium bicarbonate and 0.18 ml of methyl iodide. The solution was refluxed for 2 hours and 0.10 ml of methyl iodide was added.

An additional 0.10 ml of methyl iodide was added after 3 hours. After refluxing for an hour, the solution was evaporated to dryness *in vacuo*. The residue was extracted with hot chloroform. After evaporation of the chloroform extracts *in vacuo*, the residue was crystallized from ethanol, yield 585 mg (98%): mp 158-160°; ir ($CHCl_3$) 2960 (m), 1520 (m), 1445 (m), 1270 (s), 1168 (m), 1148 (m), and 1025 (m) cm^{-1}; nmr ($CDCl_3$) δ 3.0 (s, 9, NCH_3), 3.87 (s, 3, OCH_3), 4.52 (s, 2, CH_2N), 5.20 (s, 2, $PhCH_2$), and 6.9-7.6 (m, 8, aromatic).

Anal. Calcd. for $C_{18}H_{24}NO_2I$: C, 52.30; H, 5.81; N, 3.39. Found: C, 52.15; H, 5.79; N, 3.50.

3-Benzyloxy-4-methoxy[7S-^{3}H]benzyl acetate (74)

Silver acetate (236 mg) was added in small portions to a solution of 300 mg of (72) in 20 ml of methanol over a one hour period. After stirring for an additional 2 hours, the precipitate was filtered. The filtrate was evaporated to an oil *in vacuo*. The oil was transferred to a sublimation apparatus and sublimed at 155° (0.05 mm). The sublimed material was predominantly the desired acetate (74) and crystallized from benzene-hexane, yield 107 mg (52%): mp 95-97°; ir ($CHCl_3$) 1740 (s) (C=O), 1520 (s), 1365 (m), 1255 (s), 1160 (m), 1138 (s), and 1030 (s) cm^{-1}; nmr ($CDCl_3$) δ 2.08 (s, 3, CH_3CO), 3.83 (s, 3, OCH_3), 4.95 (s, 2, $PhCH_2$), 5.10 (s, 2, CH_2OAc), and 6.7-7.5 (m, 8, aromatic).

M.S. Calcd. for $C_{17}H_{18}O_3$: 286.1205. Found: 286.1197.

3-Benzyloxy-4-methoxy[7S-^{3}H]benzyl alcohol (75)

The acetate (74) (100 mg) was dissolved in 20 ml of dry diethyl ether and stirred for 2 hours with 50 mg of lithium aluminum hydride. The excess hydride was destroyed by adding 20 ml of saturated sodium potassium tartrate solution. The layers were separated and the aqueous solution was extracted with diethyl ether. The ether extracts were combined, washed with saturated sodium chloride solution, dried with sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was crystallized from benzene-hexane, yield 57 mg (64%): mp 72-73° (lit. (39) 76°); (^{3}H - 5.54 x 10^8 dpm/mmol). The material was identical with an authentic sample by TLC in chloroform and in ethyl acetate.

Oxidation of (75)

A solution of (75) (27 mg) in 1.5 ml of acetone was added to a mixture of distilled water (25 ml), 5 ml of glycine buffer (0.05 N, pH 8.8), 50 mg of NAD, 3 N sodium hydroxide (a few drops to adjust the pH to between pH 8 and 9), and 10 mg of LADH at 37.5° in 0.5 ml aliquots at one-half hour intervals. After 4 hours, an additional 10 mg of LADH was added to the mixture. After two hours, TLC of a small aliquot of the reaction mixture indicated that only

a trace of the starting alcohol (75) remained. The reaction mixture was extracted with ethyl acetate. The ethyl acetate solution was concentrated in vacuo and chromatographed on silica gel TLC plates developed in chloroform. The aldehyde (76) (22 mg) and the alcohol (75) (3 mg) were recovered from the plates. The aldehyde (76) was crystallized from ether-hexane to give white crystals, mp 57-60° (lit. (67) 60-61°); (^{3}H - 3.79 x 10^8 dpm/mmol). The ratio of the specific activity of the aldehyde (76) to that of the starting alcohol (75) was 68.5%. A solution of the aldehyde (76) (10 mg) in 5 ml of dioxane was oxidized with 10 mg of potassium permanganate in 2 ml of water. The reaction was worked up as previously described on page 74. The 3-benzyloxy-4-methoxybenzoic acid (77) was crystallized from ethanol, yield 6 mg (56%): mp 177-178° (lit. (68) 177°); (^{3}H - 1.82 x 10^4 dpm/mmol).

O-Methyl[1-^{14}C]norbelladine hydrochloride (78)

O-Methyl[1-^{14}C]norbelladine hydrochloride (78) was synthesized by the method of Barton and coworkers (18). It was crystallized from ethanol-ether to yield white fluffy crystals, mp 204-207° (lit. (18) 205-207°).

O-Methyl[1'R-^{3}H,1-^{14}C]norbelladine hydrochloride (79)

O-Methyl[1'R-^{3}H]norbelladine hydrochloride (71) and O-methyl[1-^{14}C]norbelladine hydrochloride (78) were combined

and crystallized from ethanol-ether to constant activity (^{3}H - 22.1 μci/mg; ^{14}C - 15.1 μci/mg): mp 204-207° (lit. (18) 205-207°). It was identical by comparative TLC in solvents B and C with an authentic sample.

Synthesis of 3-Hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine Hydrochloride

3-Benzyloxy-4-methoxy[7-^{3}H]benzyl alcohol (80)

A solution of the aldehyde (66) (300 mg) in 20 ml of methanol was stirred for three hours with 114 mg of sodium borohydride. The solvent was evaporated *in vacuo* and the residue was dissolved in a two phase system of water and ethyl acetate. The layers were separated. The ethyl acetate layer was dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The product was crystallized from benzene-hexane, yield 293 mg (96%): mp 72-73° (lit. (39) 76°). The product was identical by comparative TLC in chloroform and in ethyl acetate with authentic material.

3-Benzyloxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (81)

3-Benzyloxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (81) was prepared from (80) in the same fashion as previously described for the synthesis of 3-benzyloxy-4-methoxy[7R-^{3}H]-benzylamine hydrochloride (68): mp 245-246°.

3-Hydroxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (82)

A solution of the benzyloxyamine hydrochloride (81) (40 mg) in 15 ml of methanol was stirred with 50 mg of 10% Pd on charcoal under a hydrogen atmosphere. After the absorption of the theoretical amount of hydrogen, the catalyst was filtered from the methanol solution and the solution was evaporated to dryness in vacuo. The product was crystallized from ethanol-ether, yield 24 mg (96%): mp 176-177°; ir (KBr) 3400 (m), 3090 (s), 1530 (s), and 1280 (m) cm^{-1}; nmr (d_4-methanol) δ 3.85 (s, 3, OCH_3), 3.95 (s, 2, CH_2N), 4.87 (s, 4, NH_3, OH), and 6.97 (s, 3, aromatic).

M.S. Calcd. for $C_8H_{11}NO_2$: 153.0788. Found: 153.0790.

3-Benzyloxy-4-methoxy[7-^{14}C]benzoic acid (85)

3-Benzyloxy-4-methoxy[7-^{14}C]benzoic acid (85) was prepared by carbonation of 3-benzyloxy-4-methoxyphenylmagnesium bromide with $^{14}CO_2$ as described by Chan (39). The product was crystallized from ethanol: mp 177-179° (lit. (68) 177°).

3-Benzyloxy-4-methoxy[7-^{14}C]benzyl alcohol (86)

The alcohol (86) was prepared by reduction of the acid (85) with lithium aluminum hydride as described by Chan (39): mp 72-73° (lit. (39) 76°).

3-Hydroxy-4-methoxy[7-^{14}C]benzylamine hydrochloride (88)

The amine hydrochloride (88) was prepared from 3-benzyloxy-4-methoxy[7-^{14}C]benzyl alcohol (86) in the same manner that 3-hydroxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (82) was prepared from (80). The product was crystallized from ethanol-ether, mp 176-177°.

3-Hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (92)

3-Hydroxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (82) and 3-hydroxy-4-methoxy[7-^{14}C]benzylamine hydrochloride (88) were combined and recrystallized from ethanol-ether to constant activity (^{3}H - 20.1 μci/mg; ^{14}C - 20.5 μci/mg): mp 176-177°.

Synthesis of N-Methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine Hydrochloride

3-Benzyloxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (83)

A mixture of the aldehyde (66) (50 mg), 100 mg of methylamine hydrochloride and 50 mg of sodium bicarbonate in 2 ml of i-propyl alcohol was refluxed in a nitrogen atmosphere for two hours. After cooling to room temperature, sodium borohydride (100 mg) was added and the reaction mixture was stirred for four hours at room temperature. The solvent was evaporated in vacuo and the residue was dissolved in 25 ml of 10% hydrochloric acid. The aqueous acid solution was washed with diethyl ether and the latter was discarded.

After neutralization with concentrated ammonium hydroxide to pH 8, the aqueous solution was extracted with diethyl ether. The ether solution was washed with saturated sodium chloride solution, dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The product was converted to its hydrochloride salt and crystallized from ethanol-ether, yield 23.6 mg (39%): mp 214-216° (lit. (71) 212°).

N-Methyl-3-hydroxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (84)

The amine hydrochloride (83) was hydrogenated in the same fashion as previously described for the preparation of 3-hydroxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (82). The product was crystallized from ethanol-ether, yield 16.2 mg (98%): mp 167-169° (lit. (18) 171-172°).

O-Benzyl[7-^{14}C]isovanillin (89)

The aldehyde (89) was prepared by oxidation of the benzyl alcohol (86) with manganese dioxide as described by Chan (39). The product was crystallized from benzene-hexane, yield 88.2 mg (100%): mp 57-60° (lit. (67) 60-61°).

N-Methyl-3-hydroxy-4-methoxy[7-^{14}C]benzylamine hydrochloride (91)

The amine hydrochloride (91) was prepared from O-benzyl-[7-^{14}C]isovanillin (89) in the same manner as that previously

described for the synthesis of N-methyl-3-hydroxy-4-methoxy-[7-^{3}H]benzylamine hydrochloride (84). The product was crystallized from ethanol-ether: mp 167-169° (lit. (18) 171-172°).

N-Methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (93)

N-Methyl-3-hydroxy-4-methoxy[7-^{3}H]benzylamine hydrochloride (84) and N-methyl-3-hydroxy-4-methoxy[7-^{14}C]-benzylamine hydrochloride (91) were combined and recrystallized to constant activity (^{3}H - 16.0 μci/mg; ^{14}C - 12.3 μci/mg): mp 168-169° (lit. (18) 171-172°).

Degradation of 3-Hydroxy-4-methoxy-[7-^{3}H,^{14}C]benzylamine Hydrochloride and N-Methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]-benzylamine Hydrochloride

The amine hydrochlorides (92) and (93) were diluted with the corresponding non-radioactive amine hydrochlorides and degraded by conversion to the corresponding veratryl-amines (94) and (95), which were than oxidized to veratric acid (96). The results for these degradations are summarized in Tables 6 and 7.

Veratrylamine hydrochlorides (94) and (96)

The diluted amine hydrochloride ((92) or (93)) (100 mg) was dissolved in 10 ml of methanol and a ten-fold excess of

Table 6. Degradation of 3-hydroxy-4-methoxy[7-^{3}H,^{14}C]-benzylamine hydrochloride (92)

Compound	Amount (mg)	Specific activity (dpm/mmol)	Isotope	$^{3}H/^{14}C$ Ratio
[7-^{3}H,^{14}C]veratryl-amine hydro-chloride (94)	75	1.24×10^{7} 1.29×10^{7}	^{3}H ^{14}C	0.96
Veratric acid (96)	56	3.38×10^{6} 1.24×10^{7}	^{3}H ^{14}C	0.26

Table 7. Degradation of N-methyl-3-hydroxy-4-methoxy-[7-^{3}H,^{14}C]benzylamine hydrochloride (93)

Compound	Amount (mg)	Specific activity (dpm/mmol)	Isotope	$^{3}H/^{14}C$ Ratio
N-Methyl[7-^{3}H,^{14}C]-veratrylamine hydrochloride (95)	75	2.15×10^{7} 1.69×10^{7}	^{3}H ^{14}C	1.27
Veratric acid (96)	49	0.00 1.65×10^{7}	^{3}H ^{14}C	———

ethereal diazomethane was added. After standing for 12 hours, the reaction mixture was evaporated to approximately 10% of its original volume. The reaction mixture was diluted with diethyl ether and extracted with a 10% sodium hydroxide solution. The ether solution was washed with a saturated sodium chloride solution, dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The product was converted to the hydrochloride and crystallized from ethanol-ether, yield 101 mg (95%). The melting point of the [7-^{3}H,^{14}C]-veratrylamine hydrochloride (94) was 257-259° (lit. (72) 257-258°) and of N-methyl[7-^{3}H,^{14}C]veratrylamine hydrochloride (95) was 213-214° (lit. (73) 207°).

Veratric acid (96)

To a solution of 75 mg of the veratrylamine hydrochloride ((94) or (95)) in 5 ml of water, whose pH had been adjusted to pH 9 with potassium carbonate, was added 300 mg of potassium permanganate. The reaction mixture was heated for one hour on a steam both and then sulfur dioxide was bubbled in until the manganese dioxide precipitate disappeared. The pH of the reaction mixture was adjusted to pH 5 with concentrated sulfuric acid. The aqueous solution was extracted with chloroform. The chloroform extract was in turn extracted with a 10% sodium hydroxide solution. The sodium hydroxide solution was cooled,

acidified with concentrated sulfuric acid, and extracted with chloroform. The chloroform solution was dried with sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The product was crystallized from water, yield 56 mg (85%): mp 180-182° (lit. (74) 181°).

Zeisel degradation

The Zeisel degradation was carried out on 25 mg of [7-^{3}H,^{14}C]veratrylamine hydrochloride (94) (^{3}H - 1.24 x 10^{7} dpm/mmol; ^{14}C - 1.29 x 10^{7} dpm/mmol) by the method described by Miller (11) and by Feinstein (7). The methyltriethylammonium iodide was crystallized from methanol-ether, yield 12 mg: mp 277-279° (lit. (11) 277-280°). The iodide salt contained neither the tritium label nor the carbon-14 label.

Feeding Experiments

Administration of labeled precursors to the plant

The doubly-labeled precursors were purified and fed as the hydrochloride salt. A portion of each precursor was weighed and dissolved in 0.5 to 1.0 ml of water (pH 6) and introduced into the bulb with a fine hypodermic needle. The plants were grown in a fume hood under artificial light for a period of three weeks. The bulbs were harvested and processed. The bulbs were obtained from International Growers Exchange, Inc., Farmington, Michigan.

The isolation of the alkaloid fractions was carried out in the same manner and, in the interest of brevity, only the general procedure will be described below.

The bulbs (400 to 700 g) were macerated in a Waring Blendor with 95% ethanol (2.5 l.), left to stand overnight, and filtered. The solid material was extracted an additional two times with 95% ethanol (2 x 1.5 l.). The combined ethanol extracts were evaporated to about 1 l. under reduced pressure, acidified to pH 4 with concentrated hydrochloric acid, and filtered. The aqueous solution was extracted with benzene (4 x 200 ml) to remove neutral substances. Extraction with chloroform gave the crude chloroform-soluble hydrochlorides. The solution was then basified to pH 8-9 with ammonium hydroxide and extracted with chloroform (4 x 200 ml) to give the pH 8 "bases". The aqueous fraction was adjusted to pH 10 and extracted with chloroform to give the pH 10 "bases".

A chloroform solution of the chloroform-soluble hydrochlorides was washed with 10% sodium hydroxide solution to give the chloroform-soluble hydrochloride free "bases". The chloroform solution was dried with sodium sulfate, filtered, and evaporated to dryness in vacuo.

The chloroform solution of the pH 8 "bases" was extracted with 10% sodium hydroxide solution to remove the phenolic "bases". The chloroform solution of the

non-phenolic pH 8 "bases" was dried with sodium sulfate, filtered, and concentrated in vacuo. The basic solution containing the phenolic "bases" was neutralized to pH 8-9 with hydrochloric acid and extracted with chloroform. The chloroform solution was dried with sodium sulfate, filtered, and evaporated in vacuo to give the phenolic "bases".

The chloroform solution of the pH 10 "bases" was dried with sodium sulfate, filtered, and evaporated to dryness in vacuo. The non-phenolic pH 8 "bases" and the pH 10 "bases" were combined.

The data for the crude "bases" is summarized in Tables 8, 9, 10, and 11.

Isolation of the alkaloids from the Crinum powellii feeding experiment

The non-phenolic pH 8 and pH 10 "bases" were dissolved in chloroform and allowed to stand overnight. The crude lycorine was filtered from the chloroform solution and crystallized as its hydrochloride salt from ethanol-water: mp 212-214° (lit. (1) 215-216°). The filtrate was evaporated and separated by preparative thick layer chromatography in solvent B. The band at R_f=0.65 was removed and crinamine was recovered from the silica gel. Crinamine was crystallized from acetone: mp 198-200° (lit. (75) 198-199°). The band at R_f=0.55 was rechromatographed in solvent C on

Table 8. Crude alkaloid fractions for the feeding of O-methyl[1'R-^{3}H,^{14}C]-norbelladine hydrochloride (79) to *Crinum erubescens*[a]

Alkaloid Fraction	Amount (gram)	Total activity (μci)	Per cent incorporation	Isotope	$^{3}H/^{14}C$ Ratio
Chloroform-soluble hydrochloride free "bases"	0.814	14.6 9.6	9.4 9.05	^{3}H ^{14}C	1.51
Phenolic "bases"	0.046	39.4 22.6	25.0 21.0	^{3}H ^{14}C	1.74
Non-phenolic pH 8 and pH 10 "bases"	1.40	36.8 19.7	23.0 18.5	^{3}H ^{14}C	1.87

[a]Weight of wet bulbs - - - - - 438 grams.

Table 9. Crude alkaloid fractions for the feeding of O-methyl[1'R-^{3}H,1-^{14}C]-norbelladine hydrochloride (79) to Nerine bowdenii[a]

Alkaloid Fraction	Amount (gram)	Total activity (μci)	Per cent incorporation	Isotope	^{3}H/^{14}C Ratio
Chloroform-soluble hydrochloride free "bases"	1.089	7.74 5.15	4.5 4.4	^{3}H ^{14}C	1.50
Phenolic "bases"	0.204	51.2 35.1	29.6 29.8	^{3}H ^{14}C	1.43
Non-phenolic pH 8 and pH 10 "bases"	1.601	5.19 4.2	3.0 3.5	^{3}H ^{14}C	1.24

[a]Weight of wet bulbs - - - - - 761 grams.

Table 10. Crude alkaloid fractions for the feeding of 3-hydroxy-4-methoxy[7-^{3}H, ^{14}C]benzylamine hydrochloride (92) to Nerine bowdenii[a]

Alkaloid Fraction	Amount (gram)	Total activity (μci)	Per cent incorporation	Isotope	^{3}H/^{14}C Ratio
Chloroform-soluble hydrochloride free "bases"	0.342	0.61 0.66	0.46 0.48	^{3}H ^{14}C	0.92
Phenolic "bases"	0.066	0.00 0.00	—— ——	—— ——	——
Non-phenolic pH 8 and pH 10 "bases"	0.823	—— ——	—— ——	—— ——	——

[a]Weight of wet bulbs - - - - - 434 grams.

Table 11. Crude alkaloid fractions for the feeding of N-methyl-3-hydroxy-4-methoxy[7-^{3}H,^{14}C]benzylamine hydrochloride (93) to Nerine bowdenii

Alkaloid Fraction	Amount (gram)	Total activity (μci)	Per cent incorporation	Isotope	$^{3}H/^{14}C$ Ratio
Chloroform-soluble hydrochloride free "bases"	0.754	0.17 0.08	0.12 0.07	^{3}H ^{14}C	2.12
Phenolic "bases"	0.097	0.38 0.32	0.28 0.31	^{3}H ^{14}C	1.19
Non-phenolic pH 8 and pH 10 "bases"	1.317	27 24	20 23	^{3}H ^{14}C	1.09

[a]Weight of wet bulbs - - - - - 494 grams.

silica gel plates. The band at R_f=0.6 was recovered from the silica gel and proved to be 6-hydroxycrinamine. 6-Hydroxycrinamine was crystallized from acetone-ether: mp 212-213° (lit. (1) 210°).

The amounts isolated and the incorporation data for each alkaloid is summarized in Table 2.

Isolation of the alkaloids from the Nerine bowdenii feeding experiments

The non-phenolic pH 8 and pH 10 "bases" were dissolved in chloroform and allowed to stand overnight. The crude lycorine was filtered from the chloroform solution and crystallized as its hydrochloride salt from ethanol-water: mp 212-214° (lit. (1) 215-216°). The filtrate was evaporated and separated by preparative thick layer chromatography in solvent B. The band at R_f=0.6 was scraped off and the alkaloidal material was recovered from the silica gel. The band at R_f=0.4 provided ambelline. The crude ambelline was crystallized from ethyl acetate: mp 259-261° (lit. (1) 260-261°). The band at R_f=0.35 provided crinamidine. The crude crinamidine was crystallized from acetone: mp 227-230° (lit. (76) 228-230°). The band at R_f=0.6 was rechromatographed on silica gel plates in solvent C. The band at R_f=0.6 was scraped off and 6-hydroxybuphanidrine was recovered. Repeated attempts to crystallize 6-hydroxybuphanidrine failed. The 6-hydroxybuphanidrine was converted

to its methiodide salt by the method of Slabaugh (77) and was crystallized from methanol-ether: mp 242-244°; mmp 241-244° (lit. (77) 240-242°).

The chloroform-soluble free "bases" were chromatographed by preparative thick layer chromatography in solvent B. The band at R_f=0.8 provided belladine. The crude belladine was converted to its hydrochloride salt and crystallized from ethanol-ether: mp 191-193° (lit. (76) 190-192°). The band at R_f=0.6 was scraped off and undulatine was recovered from the silica gel. The crude undulatine was crystallized from ethanol-water: mp 150-151° (lit. (76) 149-150°).

The amounts of each alkaloid isolated and the incorporation data for the feeding experiments with Nerine bowdenii are summarized in Tables 3, 4, and 5.

6-Oxocrinamine (100)

The isolated 6-hydroxycrinamine was diluted with non-radioactive material. The diluted 6-hydroxycrinamine (100 mg) (^3H - 8.65 x 10^4 dpm/mmol; ^{14}C - 9.60 x 10^4 dpm/mmol) was dissolved in 20 ml of dry chloroform and stirred for 45 minutes with 1 g of freshly prepared manganese dioxide (62). The manganese dioxide was filtered from the solution and the solution was evaporated to dryness in vacuo. The residue was separated by preparative thick layer chromatography in solvent A. The band containing 6-oxocrinamine (100) was

recovered. The crude 6-oxocrinamine (100) was crystallized from ethyl acetate, yield 15 mg (15%): mp 195-196°; mmp 194-196° (lit. (78) 195-196°); (^{3}H - 0.00; ^{14}C - 9.83 x 10^{4} dpm/mmol).

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